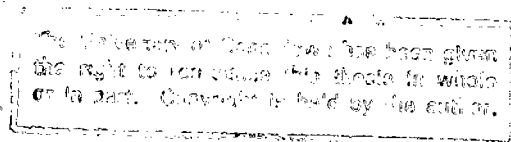


THE CROSSED MESOSTRIATAL PATHWAY AND CIRCLING BEHAVIOUR IN RATS.

Submitted in fulfilment of the requirements for the degree of
Master of Science.

Gerhard Derek Van Wageningen B.Sc Hons.

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Observatory 7925
Cape Town
South Africa
March 1987



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For Gerhard, Geraldine, and Anne.

CONTENTS:	PAGE
ABSTRACT	1
ACKNOWLEDGEMENTS	5
GLOSSARY OF ABBREVIATIONS	6
1.0 INTRODUCTION	7
<hr/>	
1.1 DEFINITION OF THE PROBLEM AND OUTLINE OF THE THESIS	8
1.2 THE UNGERSTEDT ROTATING RAT MODEL	12
1.3 THE JERUSSI-GLICK ROTATING RAT MODEL	17
1.4 CIRCLING AND HEMISPHERICAL DOMINANCE	19
1.5 THE ANATOMICAL SUBSTRATE OF CIRCLING	24
1.5.1 SNR AS AN OUTPUT STATION FOR BASAL GANGLIA FUNCTION RELEVANT TO CIRCLING	25
1.5.2 NON-DOPAMINERGIC CIRCLING	30
1.5.3 BASAL GANGLIA OUTPUT VIA THALAMUS	38
1.5.4 BASAL GANGLIA OUTPUT VIA THE MIDBRAIN TECTAL AND TEGMENTAL REGIONS	41
1.5.5 SYNOPSIS	52
1.6 THE NIGROSTRIATAL DOPAMINE SYSTEM	56
1.7 THE PHENOMENON OF FUNCTIONAL INTERDEPENDENCE BETWEEN THE NIGROSTRIATAL SYSTEMS	73
1.8 NEURAL SUBSTRATES FOR INTERHEMISPHERIC COMMUNICATION OF THE NIGROSTRIATAL SYSTEMS	75
1.9 THE CROSSED MESOSTRIATAL PROJECTION	78
1.10 THE APPLICATION OF ROTATING RAT MODELS TO BASAL GANGLIA PATHOLOGIES	84

2.0 GENERAL METHODS	89
<hr/>	
2.1 ANIMAL BEHAVIOUR AS AN INDEX OF THE PERFORMANCE OF NEUROCHEMICALLY AND ANATOMICALLY DISTINCT BRAIN AREAS	90
2.2 MONITORING ROTATIONAL BEHAVIOUR	93
2.2.1 THE ROTOMETERS	94
2.2.2 DATA LOGGING	95
2.2.3 COMPUTER PROGRAMS	99
2.3 GENERAL SURGICAL METHODS	111
2.3.1 THE HRP PROCEDURE	112
2.3.2 6-OHDA LESIONING	112
2.4 HISTOLOGY	115
2.4.1 NEUROANATOMICAL TRACT TRACING WITH HRP	115
2.4.2 HRP HISTOCHEMISTRY	122
2.4.3 CRESYL VIOLET CHROMATIN STAINING PROCEDURE	127
3.0 HRP INVESTIGATION OF THE CROSSED MESOSTRIATAL SYSTEM	129
<hr/>	
3.1 INTRODUCTION	130
3.2 CONFIRMING THE CROSSED PROJECTION, ESTABLISHING ITS EXTENT AND SITE OF DECUSSATION	131
3.2.1 CONTROLS	131
3.2.2 NORMALISATION RATIONALE	141
3.2.3 EVIDENCE FOR THE SITE OF DECUSSATION	147
3.3 DISCUSSION	164

4.0 THE EFFECT ON CIRCLING BEHAVIOUR OF STANDARD AND RESTRICTED

LESIONS OF THE VENTRAL MESENCEPHALIC DOPAMINERGIC CELL GROUPS

4.1 INTRODUCTION	172
4.2 MATERIALS AND METHODS	173
4.3 RESULTS	177
4.3.1 STANDARD AND RESTRICTED LESIONS OF THE SN	177
4.3.2 THE EFFECT OF STANDARD LESIONS OF THE SN ON CIRCLING BEHAVIOUR	179
4.3.3 RESTRICTED LESIONS OF THE LATERAL ASPECT OF SN	190
4.3.4 RESTRICTED LESIONS OF THE VENTRAL TEGMENTAL DECUSSATION	205
4.3.5 RESTRICTED LESIONS OF THE VENTRAL SN	215
4.3.6 RESTRICTED LESIONS OF THE ANTEROMEDIAL SN	225
4.3.7 INVESTIGATION OF THE CROSSED MESOSTRIATAL PROJECTION AS A FUNCTION OF RECOVERY FROM MOTOR ASYMMETRY	276
4.4 DISCUSSION AND CONCLUSIONS	282
5.0 GENERAL DISCUSSION AND CONCLUSIONS	290
<hr/>	
5.1 INTRODUCTION	291
5.2 ROTATING RAT MODELS	293
5.3 THE CROSSED MESOSTRIATAL PROJECTION	300
5.4 THE EFFECT OF STANDARD AND RESTRICTED LESIONS OF THE VENTRAL MESENCEPHALON ON ROTATIONAL BEHAVIOUR	304
5.5 RECOVERY FROM ROTATIONAL ASYMMETRY	312
5.6 SUMMARY AND CONCLUSION	321

REFERENCES	325
APPENDIX 1 INTERRUPT GENERATOR	346
APPENDIX 2 PHOTODETECTORS AND SCHMIDT TRIGGERS	348
APPENDIX 3 TABLES OF CONTROL CROSSED PROJECTION ANIMALS	350
APPENDIX 4 SENSORY-MOTOR DESCRIPTION OF #254-#277 FOLLOWING 6-OHDA LESION	362

ABSTRACT:

THE CROSSED MESOSTRIATAL PATHWAY AND CIRCLING BEHAVIOUR IN RATS.

(6-hydroxydopamine)

Rats with unilateral 6-OHDA[^] lesions of the nigrostriatal (NS) projection display motor asymmetry in the form of rotational behaviour. The rotation is in the direction ipsilateral with respect to the lesioned side (Ungerstedt 1979). The net ipsilateral rotations decrease with time, from 1 week to about a month. This decrease has been interpreted as recovery from the lesion-induced motor asymmetry (Glick and Cox 1978). Pritzel et al. (1983) have ascribed the recovery from motor asymmetry to increased activity of a crossed NS projection, which is spared by the ipsilateral lesion. The present study has defined the size and anatomical path of this crossed projection, and has examined its involvement in the behavioural recovery of rats from lesion-induced motor asymmetry.

The anatomy of the crossed projection was investigated in male Long-Evans rats using retrograde HRP tract tracing from deposition sites in the striatum. The size of the crossed projection was found to be between 0.39% and 3.61% ($1.86 \pm 1.04\%$, SEM, $n=11$) of the ipsilateral projection. 79.6% of the contralateral cells lay between AP=2.4 and AP=1.0. Most (82.3%) of these contralateral cells fell within the ventral tegmental decussation (DTV) and ventral tegmental area of Tsai (VTN). The remainder were seen within the medial substantia nigra pars compacta (SNC). Thus, the somata of the crossed projection are not restricted to the SN. They are widely distributed in the ventral mesencephalon. Consequently we will use the name 'crossed mesostriatal projection' rather than 'crossed nigrostriatal projection'.

Knowledge of the site of decussation of the crossed projection was needed in order to place a restricted lesion of the ventral mesencephalon such that it induced ipsilateral rotation while sparing the crossed projection. Thus, we have used mechanical and chemical lesions to establish the site of decussation of the crossed projection, by evaluating the effect of the lesions on retrograde HRP labelling of the contralateral somata. In order to facilitate inter-animal comparison a contralateral index (CI) was calculated which normalised the number of contralateral labelled somata. Thus $CI=0$ indicated the absence of the crossed projection, while $CI>0$ indicated its presence and extent. CI varied from 1.9 to 16.1 for control animals (5.8 ± 4 , SEM, $n=11$). Midthalamic transection left the distribution of crossed cells unchanged ($CI=3.61$ to 14.85 , $n=5$). However, mesencephalic transection produced total disruption of the crossed projection ($CI=0$, $n=5$). Discrete 6 hydroxydopamine (6-OHDA) lesioning of the DTV produced a marked attenuation of the crossed cells ($CI=0$ to 1.52 , $n=4$). It is concluded that the crossed mesostriatal projection decussates via the DTV.

The rotational behaviour of the animals was measured in an automated rotometer. This enabled three parameters of rotation to

be generated. (a) Total rotations: the number of turns made, without regard to direction. This is a measure of the animal's general motor activity level. (b) Nett rotations: the number of turns made towards the dominant side. This represents a composite of activity and asymmetry of motor activity. (c) The asymmetry index: $AI = \text{nett} / \text{total} * 100$. Behavioural recovery was examined in relation to unilateral 6-OHDA lesions of the NS projection. Recovery was assessed by comparison of nett rotations, total rotations, and AI, on the final day of testing post lesion with respect to the first. The criterion for recovery encountered in the literature is decreased nett rotations. In this study, however, the other parameters were also examined for recovery. This enabled us to specify whether recovery was from asymmetry or hyperactivity.

Standard (5ul, 10ug 6-OHDA) lesions of the dominant SN induced rotation ipsilateral to the lesioned side. Amphetamine (1mg/Kg, i.p.), a dopamine releaser and uptake inhibitor, accentuated this effect. Apomorphine (1mg/Kg, i.p.), a dopamine agonist, caused rotation contralateral to the lesioned side. These results confirm those generally reported (Ungerstedt 1971, Glick et al. 1976). There was no recovery from spontaneous nett or total rotations. However, there was significant recovery from amphetamine (1mg/Kg, i.p.) induced total rotations ($p=0.02$, $n=6$). AI was not significantly changed over the test period for either spontaneous or amphetamine induced rotation. Since our animals recovered from amphetamine induced hyperactivity only, our results do not confirm those of Pritzel et al. (1983) with respect to recovery from spontaneous rotation. Pritzel et al. (1983) demonstrated that spontaneous rotation returned to baseline levels within 1 week. In the present study, behaviour was monitored both spontaneously and with amphetamine (1mg/Kg, i.p.) for 32 days at weekly intervals. The acute phase of spontaneous rotation described by Pritzel et al. (1983) would not have been recorded in our study. We have shown that standard lesions can destroy the crossed projection in addition to the ipsilateral NS projection (Douglas et al. 1984). Thus, if recovery is mediated by the crossed projection, the location of the ipsilateral lesion is critical for the emergence of recovery. Therefore, we investigated the effect of restricted lesions of different regions of the ventral mesencephalon on circling behaviour, in order to find a lesion location which both spared the crossed projection and induced ipsilateral rotation.

66 male Long-Evans rats were screened twice pre-lesion in order to determine the hemisphere dominant for rotation. The spontaneous and amphetamine (1mg/Kg, i.p.) driven rotational behaviour in the presence of restricted lesions of the ventral mesencephalon of the dominant hemisphere was determined on days 7, 14, 24 and 32 post lesion. The restricted lesions were located in the lateral SNC ($n=15$), DTV ($n=7$), ventral SN ($n=12$), and anteromedial SNC ($n=32$). These areas are all globally affected by standard lesions. Lesions of the lateral SNC, DTV, and ventral SN did not elicit ipsilateral rotation. Only anteromedial lesions elicited ipsilateral rotation.

Nett contralateral and total amphetamine induced rotations were

significantly increased in those animals with lesions of the lateral SNC, DTV, or ventral SN. None of these lesions affected the animals' asymmetry. Animals with ventral SN lesions showed increased spontaneous nett contralateral and total rotations as well. Lateral SNC and DTV lesions did not significantly affect spontaneous behaviour. The hyperactivity induced by these lesions, reflected by increased nett contralateral and total rotations, showed no recovery over 24 days. Lateral SNC lesions spared the crossed projection, while the anatomical evidence cited earlier implies that lesions of the DTV damage the crossed projection. The ventral SN lesions did not affect the ipsilateral or crossed projections. None of these lesions induced rotation ipsilateral to the lesioned side and were thus inappropriate for studying recovery.

Since restricted anteromedial SNC lesions did evoke crossing of rotation direction from the pre-lesion direction to that ipsilateral to the lesioned side, this group of animals was used to study recovery. 18/32 animals recovered from nett ipsilateral and/or total spontaneous rotations ($18 \pm 2/43 \pm 2$ on day 7 to $7.2 \pm 0.4/12.8 \pm 0.6$ (Nett/Total, \pm SEM) on day 32 post lesion, $p < 0.05$, $p < 0.002$). Recovery from amphetamine induced rotations was seen in 14/32 animals, decreasing from $262 \pm 13/307 \pm 12.7$ to $143 \pm 7.8/154 \pm 7.5$ ($p < 0.02$). The remainder of the animals were non-recoverers, exhibiting the same or more nett ipsilateral rotations with time. The AI for the sub-group of animals recovering from nett ipsilateral rotations was unchanged on day 32 compared to day 7 post lesion for both spontaneous and amphetamine-induced rotations. Thus recovery, when it occurs, is from motor hyperactivity rather than from motor asymmetry. This recovery is similar to recovery from the amphetamine response with standard lesions, where total rotations were significantly reduced but AI was unchanged.

The role of the crossed mesostriatal projection in recovery from sensory-motor asymmetry was investigated in 19 of the animals with anteromedial lesions. HRP was infused into the striatum ipsilateral to the lesioned SN and cell counts within the lesioned and contralateral ventral mesencephalon were performed. The CI was depressed in recoverers from both spontaneous ($CI = 0.46 \pm 0.04$, SEM, $n = 11$) and amphetamine ($CI = 0.31 \pm 0.04$, SEM, $n = 9$) induced rotations. Non-recoverers had a similar CI. Thus, restricted anteromedial lesions affect the integrity of the crossed projection. Nevertheless, recovery occurred in the presence of these lesions and there was no correlation between CI and the extent of recovery. This suggests that the crossed projection did not mediate recovery. There was also no relationship between the degree of sparing of the ipsilateral SN (as assessed by HRP labelling) and recovery.

We conclude the following;

- (a) The crossed mesostriatal projection crosses in the ventral tegmental decussation.
- (b) This projection is susceptible to damage by 6-OHDA lesions of the ipsilateral SN, which are large enough, or sufficiently

critically placed to evoke a change in the dominance of rotation.
(c) Recovery is not dependent on the presence of the crossed projection. Recovery is also not dependent on the severity of the ipsilateral SN lesion. This suggests that recovery may be an extra-nigral phenomenon.

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GLOSSARY

AP Anteroposterior
AC Angular complex
AVT Area ventralis tegmenti
BC Brachium conjunctivum
CC Corpus callosum
CCK Cholecystokinin
CI Contralateral index
CL Contralateral cell counts
CPU Caudate-putamen
D Depth
DA Dopamine
DAI Change in asymmetry index
d-H₂O Distilled water
DLSC Deep layer superior colliculus
DTD Dorsal tegmental decussation
DTV Ventral tegmental decussation
EB Evans blue
GABA Gamma-amino-butyric-acid
GB Granular blue
GPi Globus pallidus internal segment
5-HT 5-Hydroxytryptamine (serotonin)
HP Habenulo-interpeduncular tract
HRP Horseradish peroxidase
hrpD Hrp density
ILC Ipsilateral cell counts
ILR Ipsilateral HRP density rating
KA Kainic acid
Kd Affinity coefficient
LAT Lateral
LHA Lateral hypothalamus
LVE Lateral vestibular nucleus
MAO Monoamine oxidase
MFB Medial forebrain bundle
MLA Midbrain locomotor area
MRF Mesencephalic reticular formation
n Number
NA Noreadrenalin
n. Acc. Nucleus accumbens
n. GC Nucleus reticularis gigante-cellularis
NS Nigrostriatal
PAG Periaqueductal grey
PC Cerebral peduncle
%D Percent depletion
%RS Percent recovery from spontaneous rotation
%RA Percent recovery from amphetamine driven rotation
PPn Pedunculopontine nucleus
RN Red nucleus
S1 Screen 1
S2 Screen 2
SC Superior colliculus
6-OHDA Six hydroxydopamine

SMD Supramamillary decussation
SN Striatonigral/substantia nigra
SNC Substantia nigra pars compacta
SND Substantia nigra pars dorsalis
SNR Substantia nigra pars reticulata
STR Striatum
2-DG 2-Deoxy-glucose
um Microns
VA-VLT Ventroanterior ventrolateral thalamus
VMT Ventromedial thalamus
VTA Ventral tegmental area
VTN Ventral tegmental nucleus of Tsai
WGA-HRP Wheat germ agglutinin HRP

EQUATIONS

$$CI = (1000 * CL) / (um * n * ILR) \quad 3.1$$

CL=contralateral labelled cell counts
um=nominal section thickness
n=number of sections
ILR=ipsilateral density rating

$$\%D = [1 - [(IC/6847.8) * (36/n) * (10 * hrp)]] * 100 \quad 3.2$$

IC=ipsilateral cell counts
6847.5=standardised control ipsilateral cell count
n=number of sections observed
36=number of 50um sections between AP=3.6 and AP=1.8
10=maximal hrp density
hrp=hrp density

$$\%R = 100 * [(a - x) / a] \quad 4.1$$

a=the number of ipsilateral nett rotations performed on day 7 post lesion
x=the number of ipsilateral nett rotations performed on day 32 post lesion

1.0 INTRODUCTION

1.1 DEFINITION OF THE PROBLEM AND OUTLINE OF THE THESIS

1.2 THE UNGERSTEDT ROTATING RAT MODEL

1.3 THE JERUSSI-GLICK ROTATING RAT MODEL

1.4 CIRCLING AND HEMISPHERICAL DOMINANCE

1.5 THE ANATOMICAL SUBSTRATE OF CIRCLING

1.5.1 SNR AS AN OUTPUT STATION FOR BASAL GANGLIA FUNCTION RELEVANT TO CIRCLING

1.5.2 NON-DOPAMINERGIC CIRCLING

1.5.3 BASAL GANGLIA OUTPUT VIA THALAMUS

1.5.4 BASAL GANGLIA OUTPUT VIA THE MIDBRAIN TECTAL AND TEGMENTAL REGIONS

1.5.5 SYNOPSIS

1.6 THE NIGROSTRIATAL DOPAMINE SYSTEMS

1.7 THE PHENOMENON OF FUNCTIONAL INTERDEPENDENCE OF THE NIGROSTRIATAL SYSTEMS

1.8 NEURONAL SUBSTRATES FOR THE INTERHEMISPHERIC COMMUNICATION OF THE NIGROSTRIATAL SYSTEMS

1.9 THE CROSSED MESOSTRIATAL SYSTEM

1.10 THE APPLICATION OF ROTATING RAT MODELS TO BASAL GANGLIA PATHOLOGIES

1.1 DEFINITION OF THE PROBLEM AND OUTLINE OF THE THESIS

Circling or rotational behaviour, as studied in rodents, is a manifestation of an asymmetrical sensory-motor transaction between the animal and its environment. The asymmetry is defined with respect to the anteroposterior axis of the animal, and has locomotor, postural (Pycock et al. 1975), and perseverance components (Lahue 1981). In addition, there is a stereotypic component to the circling syndrome which consists of repeated behaviours like grooming, gnawing, licking and sniffing. Stereotypy may be a type of perseverance. Circling occurs in normal rats, especially during their nocturnal active phase, but it can be powerfully and reliably induced and modified by manipulation of the neuronal and neurochemical status of the substantia nigra and striatum. For example, unilateral lesions of the nigrostriatal (NS) projection along its rostrocaudal length produces rotation ipsilateral to the lesioned side. This rotation is enhanced by drugs which release dopamine (DA) from terminals and/or inhibit its re-uptake (eg amphetamine). Amphetamine can induce rotation rates of the order of 500 turns per hour. Thus, circling appears to be an asymmetric behaviour congruent with striatal DA asymmetry. Striatal DA asymmetry occurs naturally and explains the natural 'handedness' of the animals. Rotation always occurs contralateral to the side of highest DAergic activity.

Circling behaviour is not, however, constant with time. Animals lesioned unilaterally within the NS system exhibit recovery from

their asymmetry and hyperactivity over a period of a few weeks (Glick and Cox 1978, Pritzel et al. 1983). Pritzel et al. (1983) demonstrated that animals recovered from spontaneous ipsilateral rotation (10 to 100 turns per hour) induced by a 6-OHDA lesion of the substantia nigra (SN) within 1 week post lesion. Glick and Cox (1978) reported recovery from both spontaneous and amphetamine driven rotational behaviour over about a month. The nature of this recovery process is not known.

Pritzel et al. (1983) suggested that recovery might be due to sprouting of a crossed component to the lesioned NS pathway. Thus, intact cells within the SN of the opposite hemisphere to that lesioned were thought to be active in compensating for the effects of the unilateral NS lesion. Alternatively, Neve et al. (1982) and Dravid et al. (1984) have suggested that recovery from lesion-induced motor asymmetry may be due to ipsilateral compensation. The cells remaining in the ipsilateral SN post lesion increase their synthesis of neurotransmitter. There are also increased receptor numbers in the striatum, the target nucleus of the NS projection. These observations suggested ipsilateral NS compensatory mechanisms which might play a role in behavioural recovery from 6-OHDA lesion-induced motor asymmetry.

In this thesis, we have examined the postulate that the crossed mesostriatal tract accounts for behavioural recovery following unilateral SN lesion. The notion that sprouting of neurones of a single tract might influence a quantifiable behaviour is attractive, since this would be a useful model for investigating

the general problem of repair mechanisms in the CNS. None of these repair processes in the CNS (eg synaptogenesis) are understood.

The aim of this study was to examine the crossed mesostriatal projection neuroanatomically, using the retrogradely transported enzyme, horseradish peroxidase (HRP). The extent of the projection and its site of decussation was specified. Furthermore, the possible role of the crossed projection in recovery from motor asymmetry induced by unilateral 6-OHDA lesions of the SN was examined.

Chapter 1 examines the historical perspective of the rotating rat model. It then describes the anatomical substrate of circling behaviour, examining the thalamocortical and midbrain routes of expression of circling behaviour, and the NS DA systems. The functional interdependence of the NS systems is highlighted and the crossed projection is described. Finally, the application and relevance of the rotating rat model to Parkinsonism is discussed.

The general methods are outlined in chapter 2. This includes a general discussion of behavioural methods. This is followed by descriptions of the monitoring procedure for measuring circling behaviour, the surgical methods and histological procedures used in the present study.

In chapter 3 the HRP investigation of the crossed mesostriatal projection is described.

Chapter 4 reports on the effect of restricted lesions of the ventral mesencephalic DAergic cell groups on circling behaviour. Lesion locations investigated are the lateral SN pars compacta, ventral SN, anteromedial SN, and ventral tegmental decussation. The role of the crossed projection in the recovery from anteromedial SNC lesions is determined.

Chapter 5 presents a general discussion and conclusions.

1.2 THE UNGERSTEDT ROTATING RAT MODEL.

The classical rotating rat model was introduced by Urban Ungerstedt (1970). In this model, a lesion of the NS system was achieved by inducing degeneration of the DA producing cells of the mesencephalon with the neurotoxin 6-hydroxydopamine (6-OHDA), presented as the dihydrobromide base in solution with ascorbate (2mg/ml) as an antioxidant. 6-OHDA was injected unilaterally into ventral mesencephalic DA neurone structures; the substantia nigra (SN; 8 ug/3ul), the rostral ventral tegmental area (VTA; 6ug/3ul), and the far lateral hypothalamus (6ug/3ul) and also caudal to the SN (8ug/4ul). The 6-OHDA lesion established the prerequisite for quantification of circling behaviour, which was realised with the use of a rotometer consisting of a hemispherical base, a cam and microswitch supported overhead. The rat was placed in the bowl and connected to the cam by a length of steel wire. The rotational behaviour was registered with electromechanical counters and plotted as turns per hour (Ungerstedt and Arbuthnott 1970).

Histological verification of the lesions using fluorescent histochemical techniques (Ungerstedt 1971) revealed lesions 0.3 to 0.5mm in diameter. Lesions located caudal to SN left DA cells and their corresponding striatal terminals intact, while lesions rostral to the SN, probably corresponding to the medial forebrain bundle (MFB), resulted in a complete disappearance of striatal terminals with partial denervation of the nucleus accumbens (n. Acc.) as well. Electrolytic lesions of the SN DA cells were also

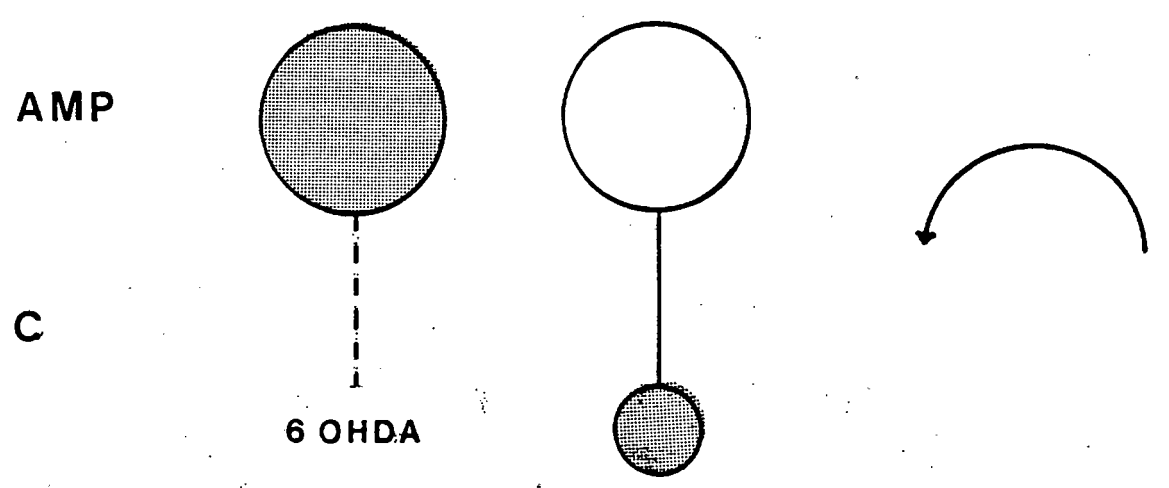
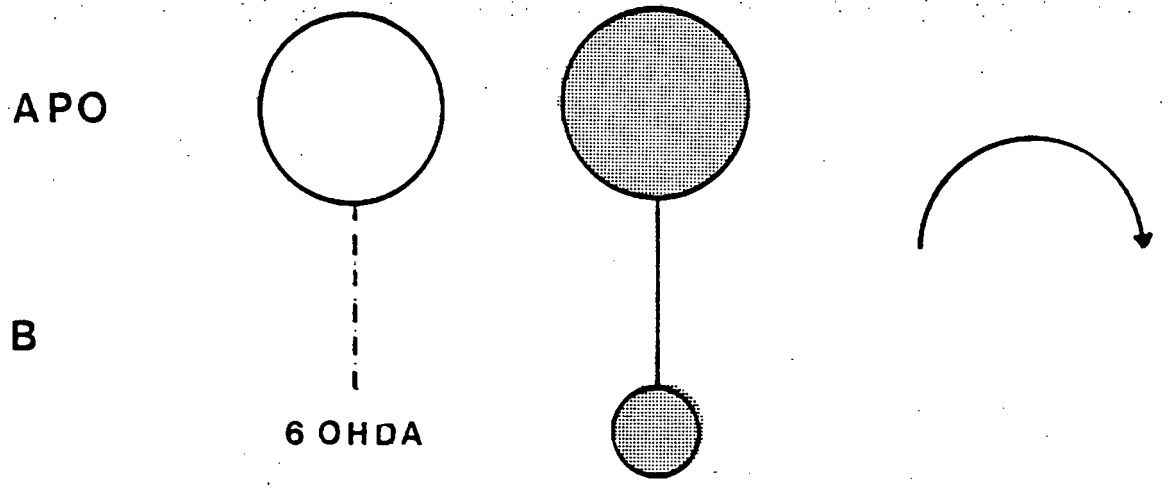
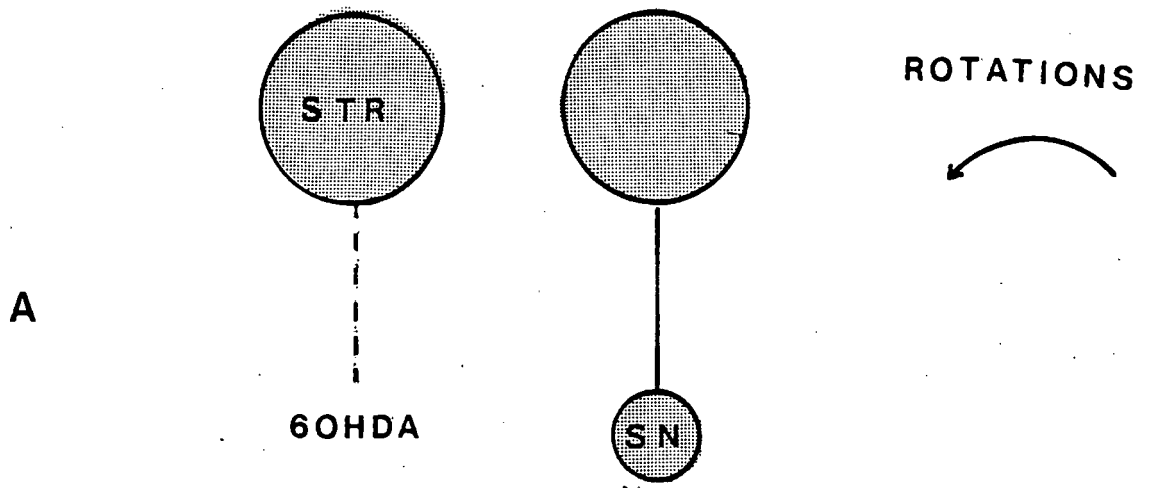
performed with similar results on striatal catecholamine histofluorescence levels. These lesions, but also 6-OHDA lesions, produced a partial disappearance of the noradrenergic (NA) neurones in the ipsilateral cortex with lateral hypothalamic lesion sites, and NA depletion of the major NA bundles with MFB lesions (Ungerstedt 1971). The animals responded to the 6-OHDA lesions in a specific and predictable way which could be enhanced by various pharmacological treatments. When animals recovered from the surgery, they rotated spontaneously towards the lesioned side, especially when stressed with a loud noise or tail clip. A postural asymmetry was also apparent with ipsilaterally directed head and tail, accompanied by contralateral limb extension indicative of contralateral sensory-motor neglect. 24 hours post lesion, the direction of spontaneous rotation had changed to that contralateral to the lesioned side, reflecting increased DA release from the degenerating NS tract. The degeneration release of DA was demonstrated by the use of the monoamine oxidase (MAO) inhibitor nialamide (100mg/Kg). Between 23 and 33 hours, in the presence of nialamide, the animal rotated contralaterally to the lesioned side reaching maximum rates of 500 turns per hour. This is due to the asymmetrical accumulation of DA in the striatum. The time course for spontaneous rotation corresponds to the time course of lesion development as revealed by histochemical studies (Hockfelt and Ungerstedt 1971). According to Ungerstedt, the spontaneous ipsilateral rotation and postural asymmetry 'soon' disappeared, though ipsilateral rotation in response to stress was still evident after two years. Amphetamine is thought to act pre-synaptically by releasing DA from the reserpine insensitive

FIG 1.1 Schematic illustration of the Ungerstedt concept of the circling rodent model. STR, striatum, SN substantia nigra, 6-OHDA, 6-hydroxydopamine lesion of the NS tract. White shading indicates activated neural substrate, while grey indicates status quo. Arrows at right indicate the direction and intensity of rotation. Rotational intensity is proportional to the length of the arrow. Amp = amphetamine, Apo = apomorphine.

A Ipsilateral circling follows a unilateral 6-OHDA lesion of the SN due to complete destruction of the ipsilateral dopaminergic cells.

B Contralateral circling following the administration of apomorphine, a dopamine receptor agonist. This is due to the activation of supersensitive dopamine receptors in the ipsilateral caudate. Circling occurs away from the side of greatest activity.

C Ipsilateral circling of greater intensity follows amphetamine administration. This is due to the DA release and uptake inhibition produced by amphetamine in the intact side.



pool of stored DA (Vane 1960, Weisman 1966, Glowinsky and Axelrod 1965, Fuxe and Ungerstedt 1968). Amphetamine (2mg/Kg) administered 30-180 minutes post SN lesion induced rotation towards the lesioned side. Between 24 and 60 hours post lesion the direction of rotation was variable. Initially contralateral, but typically 30 minutes after the amphetamine administration rotation became ipsilateral. Rotation was exclusively ipsilateral 60 hours post lesion. The events were the same for electrolytic lesions of the SN though of slightly longer duration. The dominance of rotation contralateral to the lesioned side 24 hours post lesion is due to the degradation of DA storage sites and subsequent DA release from the lysing cell membranes, and the development of post synaptic receptor supersensitivity.

There are two hypotheses of how destruction takes place at the molecular level (McGeer et al. 1979). 6-OHDA is taken up into neurones by a monoamine selective, active pumping process. In one hypothesis, hydrogen peroxide and/or superoxide radicals, generated by the in vivo oxidation of 6-OHDA, is the causative agent. In the other, rapid nucleophilic reactions of the p-quinone of 6-OHDA or other quinone reactions are responsible.

In addition to the pre-synaptic effects of DA neurone degeneration and the effect of amphetamine on DA release, there are also post synaptic consequences of 6-OHDA lesions of the NS system. The effect of apomorphine (0.25 mg/Kg), a post synaptic DA receptor agonist, was to induce rotation contralateral to the lesioned side, typically 500 turns per hour. The contralateral direction of

rotation indicated an increased sensitivity of the DA receptors in the denervated striatum. The response to apomorphine increased over the following 4 weeks, so indicating the time course of development of striatal supersensitivity (Ungerstedt 1971).

Although NA levels in the major NA bundles showed partial depletion after 6-OHDA lesions of the MFB, pharmacological evidence suggests that the NA effect on rotation is at most neuromodulatory. The decrease in NA induced by the tyrosine hydroxylase inhibitor FLA63 resulted in an increase in rotational response to amphetamine of the order of 130% of a prior amphetamine treatment. The catecholamine receptor blocker, haloperidol, abolished the response to amphetamine. Spiroperidol, a receptor blocker specific to DA receptors, also abolished the response to amphetamine in a manner identical to haloperidol (Ungerstedt 1971).

The pharmacological and lesion evidence thus implicates the NS DAergic system in the aetiology of circling behaviour. The pre-synaptically acting DA releaser, amphetamine, produces a greater activity on the intact side. Apomorphine, by activating the DA receptors on the supersensitive side, produces rotation towards the intact side. The oppositely directed rotation produced by DAergic drugs further suggests that pre- and post synaptic mechanisms are present, with rotation occurring away from the side of greatest resultant activity.

1.3 THE GLICK-JERUSSI ROTATING RAT MODEL.

Subsequent to Ungerstedt's (1971) description of rotation following unilateral lesions of the NS system (anywhere from and including the SN to the striatum), it was noted by Glick and Jerussi (1976) that unlesioned rats rotate in response to d-amphetamine sulphate (1mg/Kg), typically 92 ± 58.9 turns per hour, and apomorphine (10mg/Kg), typically 79.4 ± 63.8 turns per hour, and even spontaneously during the animal's nocturnal active phase, typically 47.1 ± 10.8 turns per hour (Glick and Cox 1978, all data quoted are mean and standard deviation). Circling behaviour in intact animals was thus postulated to be due to a natural asymmetry between the NS DA systems. This asymmetry was estimated to be of the order of 10-20% (Glick and Cox 1978). The direction of spontaneous rotation was correlated with that evoked by low doses of amphetamine (1mg/Kg), but not high doses of amphetamine (20mg/Kg), or apomorphine (10mg/Kg). The apomorphine dose used to induce circling in unlesioned animals is 2 orders of magnitude higher than that evoking circling in 6-OHDA lesioned subjects. That doses of apomorphine of 0.2mg/Kg evoke significant circling in lesioned animals 1 week post lesion is probably due to the supersensitivity of DA receptors following the lesion.

There is a large variability in the rotation of normal rats (as evidenced by the large standard deviations found by Glick and Cox 1978), and some rats (10-20%) do not rotate at all (Glick et al. 1976). However, the direction and magnitude of rotation was

consistent for each individual with time. In a group of 15 animals tested with amphetamine (1mg/Kg), 92 ± 58.9 turns per hour were recorded, and 8 days later a retest under identical conditions produced 93 ± 58.1 turns per hour. All 15 animals turned in the same direction in the 8 day test as compared to the first test.

These studies on normal rats provided further evidence for pre- and post synaptic DAergic influences in rotation. Amphetamine and apomorphine were often found to induce oppositely directed rotation in the same naive rat. Thus, amphetamine evokes pre-synaptic DA release with, for example, a circling response directed left, and apomorphine acts post synaptically on the DA-receptor complex, producing circling to the right in the same animal (Jerussi and Glick 1976). This suggests that variations in post synaptic receptor sensitivity are normally the inverse of pre-synaptic DA levels. The denervation supersensitivity following NS tract lesioning is an exaggerated example of this natural condition in which the dramatic decrease in striatal DA release results in an equally dramatic increase in striatal DA receptors in an attempt to maintain striatal DA function. A reciprocal relationship between the pre- and post synaptic mechanisms was further supported by an inverse relationship between DOPAC concentration, an index of pre-synaptic intraneuronal metabolism, and adenylate cyclase activity, the postulated DA receptor in the striatum (Jerussi et al. 1977).

1.4 CIRCLING AND HEMISPHERICAL DOMINANCE.

The original Ungerstedt model was thus modified to take pre- and post synaptic components into account, and attention was drawn to the importance of the pre-operative direction of rotation, since the two striata are not functionally equivalent. The hemisphere dominant for rotation is defined simply as the hemisphere contralateral to the direction of rotation, but it is clear from the above that the resultant quantifiable behaviour, circling, is a function of the degree of striatal DA activity which can be expressed in terms of pre- and post synaptic factors.

The choice of lesioning of the dominant or non-dominant hemisphere is important when using the 6-OHDA lesioned circling rat model as different behavioural results follow. Robinson and Becker (1983) demonstrated the importance of hemispherical dominance on amphetamine induced rotation. Of a sample of 22 SN lesioned rats, low dose amphetamine (0.75 to 1mg/Kg) was found to induce 100% ipsilateral circling following a lesion of the non-dominant side, but only 32% of subjects responded ipsilaterally following a lesion of the dominant side (Robinson and Becker 1982). Lesions were of the rostral SNC (6ug 6-OHDA in 4ul saline). The effect of dominant or non-dominant lesions on amphetamine induced circling is illustrated in fig.1.2. Fig.1.2A represents the normal intact rat in which the right NS system is dominant for rotation. Amphetamine would induce circling directed predominantly left. Following a lesion of the non-dominant side, the intrinsic asymmetry would be

FIG 1.2 Redrawn from Robinson and Becker (1982) illustrating the importance of hemispherical dominance in rotation. Circles represent relative dopamine receptor activity, while size of the straight arrow indicates intensity of dopamine for left and right hemispheres respectively. The curved arrows at right indicate the direction and intensity of rotation. Rotational intensity is proportional to the length of the arrow. The star indicates a 6-OHDA lesion of the SNC. STR, striatum, SN substantia nigra.

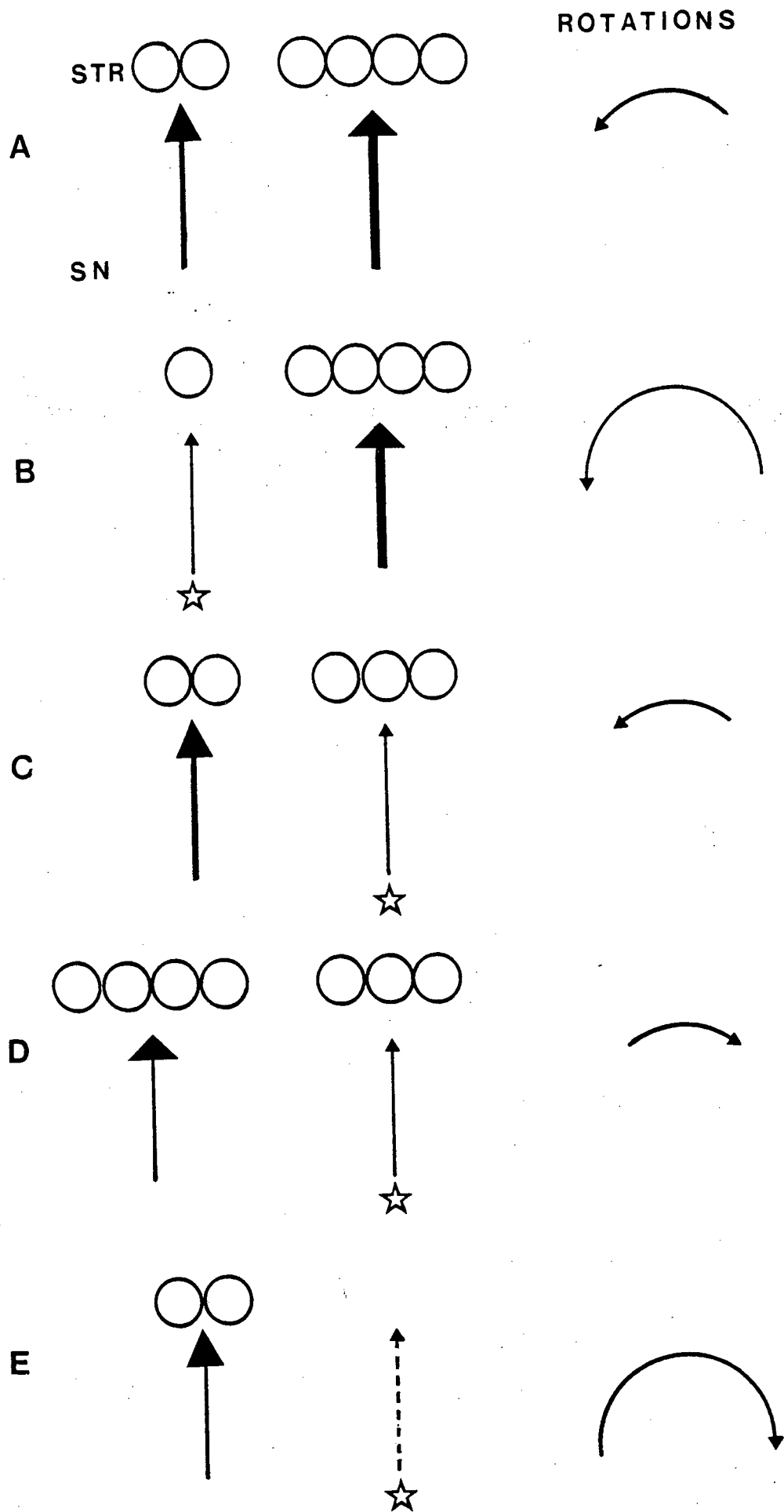
A The normal, intact rat in which the right NS system is dominant for rotation. Amphetamine induces rotation to the left.

B The non-dominant side is lesioned with 6-OHDA. The intrinsic asymmetry is enhanced, and amphetamine evokes more vigorous left rotation

C A partial lesion of the dominant SN has left a significant portion of the lesioned NS system intact. Low dose amphetamine evokes rotation contralateral to the lesioned side because the lesioned side still surpasses the non-dominant side in terms of dopamine release.

D The same lesion as 2C, but with a high dose of amphetamine. Now the non-dominant side releases more dopamine than the lesioned side, resulting in rotation ipsilateral to the lesioned side.

E A more complete lesion of the dominant SN has been performed. Low dose amphetamine evokes more dopamine release from the non-dominant side, resulting in rotation ipsilateral to the lesioned side.



enhanced and amphetamine would evoke more vigorous ipsilateral rotation (fig.1.2B). A lesion of the dominant hemisphere could lead to either ipsilateral or contralateral circling with amphetamine, depending on the degree of DA depletion resulting from the lesion. In fig.1.2C, a partial lesion of the dominant SN has left a significant proportion of the lesioned NS system intact. This residual capacity for DA production by the intact dominant NS system may still be sufficient to overdrive the intact side, especially if the animal was strongly asymmetrical to start with. A higher dose of amphetamine would increase the DA release of the unlesioned and non-dominant side such that it surpassed the partially lesioned dominant side (fig.1.2D). This assertion is supported by a decrease of the percentage contralaterally circling renegades from 63.8% to 40.3% with amphetamine at 2.6-3mg/Kg (Robinson and Becker 1982). Fig. 1.2E shows the ipsilateral rotational response to a low dose of amphetamine after a more complete lesion of the dominant hemisphere.

The degree or intensity of rotation following dominant or non-dominant lesions is also subject to variation depending on which hemisphere is lesioned. When the dominant NS system was lesioned, the resulting ipsilateral rotation was less than for a non-dominant lesion of the caudate nucleus, typically 123.3 ± 39 as compared to 242.7 ± 56.1 following an equivalent lesion of the non-dominant caudate. Circling in this case was driven with apomorphine (10mg/Kg; Jerussi and Glick 1974). In a later study, Glick and Cox (1978) found that electrolytic lesions of the dominant SN resulted in 110.1 ± 12.1 rotations compared with

212.1 \pm 14.8 following an equivalent lesion of the non-dominant side. Circling was measured 6-7 days post SN lesion in response to d-amphetamine (1mg/Kg). Similarly, 6-OHDA lesions of the dominant SN resulted in fewer (55 \pm 18.5) nett ipsilateral rotations than an equivalent non-dominant lesion (172 \pm 45.6), in response to amphetamine (1mg/Kg, Robinson and Becker 1982). With the passage of time animals with electrolytic lesions of the dominant hemisphere exhibited recovery in the direction of their original spontaneous rotation direction. Lesions of the dominant hemisphere resulted in 101.0 \pm 10.2 turns 6-7 days post lesion in the opposite direction to the pre-lesion rotation of 44.2 \pm 8.2 turns. D-amphetamine (1mg/Kg) driven rotation was still ipsilaterally directed, but attenuated from 110.1 to 74.1 with dominant sided lesions, and from 212 to 146.4 with non-dominant lesions (Glick and Cox 1978). A group of 3 animals lesioned in the non-dominant SN turned 45.3 \pm 9.1 times to their pre-lesion direction of rotation. 6 to 7 days post lesion they turned 194.1 \pm 15.1 times, and after 35 to 36 days this had decreased to 97.3 \pm 6.1.

Lesions of the nigrostriatal bundle and caudate nucleus produced a similar trend (Glick and Cox 1978).

The side chosen for lesioning was thus found to be of importance in the circling phenomenon especially when investigating recovery. Non-dominant lesions of the SN, NS bundle or caudate nucleus were found to produce an increase in the animal's natural asymmetry which was manifested as high numbers of ipsilateral rotations both spontaneously and with amphetamine. The percentage dominance (Nett

rotations/Total rotations*100) following these lesions was $95.4 \pm 0.25\%$ for the spontaneous rotation, and $99.6 \pm 0.4\%$ with amphetamine. The equivalent percentage dominance values after 35-36 days were 84.6% and 96.2%. The percentage dominance following lesions of the dominant hemisphere was $83.6 \pm 5.1\%$ for spontaneous rotation and $89.6 \pm 4.2\%$ for amphetamine-driven rotation. These values had decreased to $66.4 \pm 2.8\%$ and $83.2 \pm 3.0\%$ after 36 days respectively, and the value for spontaneous rotation, $66.4 \pm 2.8\%$, was towards the pre-lesion direction of rotation and compared to the pre-lesion percentage dominance of $71.0 \pm 4.3\%$ (Glick and Cox 1978).

Finally, there is evidence that lesions of other midbrain regions also result in rotation. Unilateral lesions of the claustrum, medial hypothalamus, mesencephalic reticular formation, medial raphe (a caudal target of the limbic striatum projection of the SN tract), locus coeruleus all induce contralateral circling. Frontal cortex ablation induces early ipsilateral rotation and contralateral rotation after 15 to 30 days. This evidence, with the exception of the medial raphe lesion effects, is ambiguous, and the reported circling may have been due to coincidental damage to the NS projection (Glick et al. 1976).

1.5. THE ANATOMICAL SUBSTRATE OF CIRCLING.

The term, "extrapyramidal system", introduced by S.A.K. Wilson (1914), has never been adequately defined anatomically. Convention over the years has made it synonymous with the basal ganglia. It is generally agreed that the term "basal ganglia" refers to the corpus striatum (striatum and pallidum), and two smaller structures, the subthalamic nucleus and the substantia nigra (CIBA 1984). However, recent evidence has shown that the synonymity between the basal ganglia and the extrapyramidal motor system is unwarranted, and that their function is more diverse and enigmatic. Increasingly sensitive neuroanatomical tract tracing techniques have revealed that the basal ganglia are highly interconnected with other parts of the brain, notably the frontal association cortices and subcortical limbic nuclei, eg amygdala and hippocampus (Brodal 1981, Szabo 1980, Beckstead et al. 1979). In addition the importance of the sensory aspect of basal ganglia function is undervalued by attempting to understand their function as that of the extrapyramidal system (Lidsky 1985). It is still not clear, however, what the neural pathways are by which the basal ganglia affect the bulbospinal motor apparatus, and thus how a behaviour originating in the basal ganglia, like circling, would be expressed.

1.5.1 SNR AS AN OUTPUT STATION FOR BASAL GANGLIA FUNCTION RELEVANT TO CIRCLING.

While the use of a DAergic-cell selective neurotoxin to lesion the ventral mesencephalon reliably resulted in ipsilateral circling with amphetamine and contralateral circling with apomorphine, it was somewhat surprising to note that electrolytic lesioning did not produce an identical result. In the latter case, ipsilateral circling was induced by both drugs (Costall 1976). This isolateral circling was postulated to be due to the simultaneous damage to the ascending DA tracts as well as to the striatonigral (SN) pathway at the level of the substantia nigra pars reticulata (SNR). The SNR was thus regarded to be an output station for striatal function of importance to circling (Di Chiara 1979).

One of the major efferent fibre tracts of the striatum is the SN projection. The SN projection is in part composed of the attenuated, unmyelinated continuation of striatal efferents that have given off co-laterals to globus pallidus at more proximal sites in their course. This suggests similarity between the SN projection and the striato-pallido-nigral projection. The topography of the SN projection is such that it projects to the entire length of the substantia nigra, preserving the mediolateral component of the striatal topology, but inverting the dorsoventral co-ordinate (Nauta and Domesick 1979, Domesick 1980, Tullock et al. 1978). GABA and substance P have been identified as SN projection neurotransmitters. The SN pathway is important in the

expression of striatally derived, DA-dependent circling, as its discrete electrolytic lesion results in ipsilateral circling with DAergic agonists like apomorphine (Garcia-Munoz 1977). The expression of circling induced by DAergic agonists in unilaterally 6-OHDA lesioned rats was also blocked by lesions of the SN pathway. Thus, the search for anatomical substrates subserving striatally derived circling behaviour centred around the SNR and its projections.

How might SNR output exert influence on the bulbospinal motor mechanisms? SNR projects to the deep layers of the superior colliculus (SC), and also innervates the mesencephalic reticular formation (MRF) adjacent to the periaqueductal grey (PAG; Rinvic et al. 1976; Beckstead et al. 1979). A projection to the pedunculopontine nucleus (PPn) has also been described (Childs and Gale 1983). The deeper strata of the SC give rise to tectobulbar and tectospinal tracts, and reticulospinal tracts originating in the medial pontomedullary reticular formation have been demonstrated (Hopkins et al. 1976, Zemlan et al. 1984). Electrical stimulation of the SNR has also been shown to decrease the firing rate of tectal cells (Chevalier et al. 1981). The basal ganglia might therefore influence the bulbospinal motor apparatus via these nigro-tectal/tegmental outputs.

Nigro-thalamic projections also exist, primarily to the ventromedial and mediodorsal nuclei (Beckstead et al. 1979, Carpenter et al. 1976). The nigro-thalamic somata are located within the dorsal region of the SNR, while the nigrotectal somata

occupy the ventral half. Evidence has, however, been presented for a projection from the centrodorsal region of the SNR to the angular complex (AC) of the midbrain (Leigh et al. 1983). This might explain some of the controversy of DA vs non-DA-dependent circling, as the nigrothalamic projections also originate from this centrodorsal region. Kilpatrick and Starr (1981) also reported haloperidol sensitive circling from a functionally defined centrodorsal region of SNR. The ventromedial thalamus projects to the plexiform layer (layer I) of the rostral two-thirds of the neocortex. This then is a route whereby the SNR can influence a wide variety of brain functions, including somatic motor mechanisms, sensory processing, and associative processing. In addition, some somata have been electrophysiologically identified to have bifurcating axons projecting to both thalamic and tectal targets (Deniau et al. 1978). In terms of information content, it is tempting to speculate that nigro-thalamic and nigro-tectal/tegmental projection sites receive similar basal ganglia modulation of their ongoing processing.

Lesioning of the SNR with kainic acid, a neurotoxin selective for glutamate responsive neurones while sparing fibre projections and DAergic cells (Olney et al. 1979), produces an acute phase during which ipsilateral circling is apparent (fig. 1.3A; DiChiara et al. 1977, Olanas et al. 1978). The acute phase is accompanied by a state of neuronal hyper-excitability and neuronal degeneration. Once the lesion is complete, contralateral circling is apparent. Treatment of such an animal with the DA receptor blocker, haloperidol, results in a potentiation of the circling behaviour.

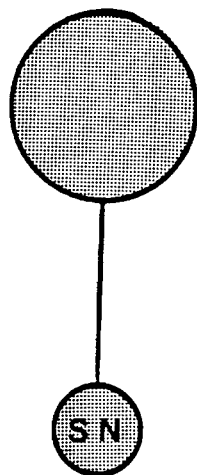
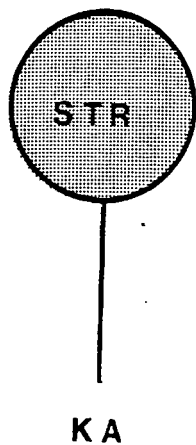
FIG 1.3. The effect of nigral manipulations on circling behaviour. Black shading indicates inhibited neural substrate, grey shading indicates status quo, white shading indicates activated neural substrate. Arrows at right indicate rotation direction and intensity. Rotation intensity is proportional to the length of the arrow. KA indicates a kainic acid lesion of the SN. STR, striatum, SN, substantia nigra. Apo = apomorphine, M = muscimol.

A The result of a kainic acid lesion of the substantia nigra pars reticulata: 1) induces an initial activation of the non-dopaminergic nigral system resulting in ipsilateral circling. Thereafter, 2) apomorphine administration results in contralateral circling due to its action on the supersensitive striatum.

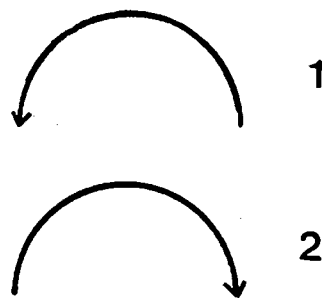
B The result of muscimol infusion into the substantia nigra pars reticulata is to induce contralateral circling. This is due to muscimol's activation of GABA receptors in the ipsilateral SN.

C Apomorphine administration concomitant with intranigral muscimol results in ipsilateral circling. This is due to the functional blockade of the apomorphine effect by the muscimol infused SN.

A

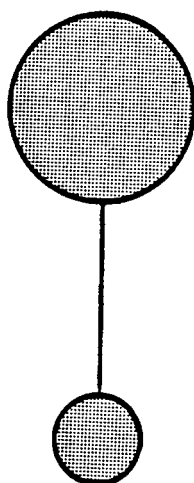
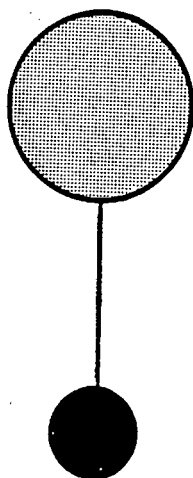


ROTATIONS



B

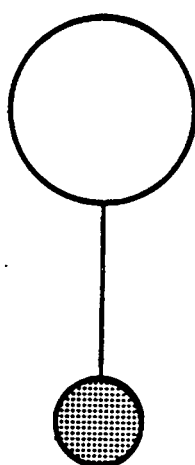
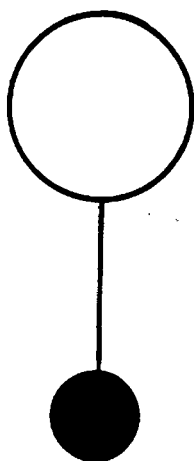
M



APO

C

M



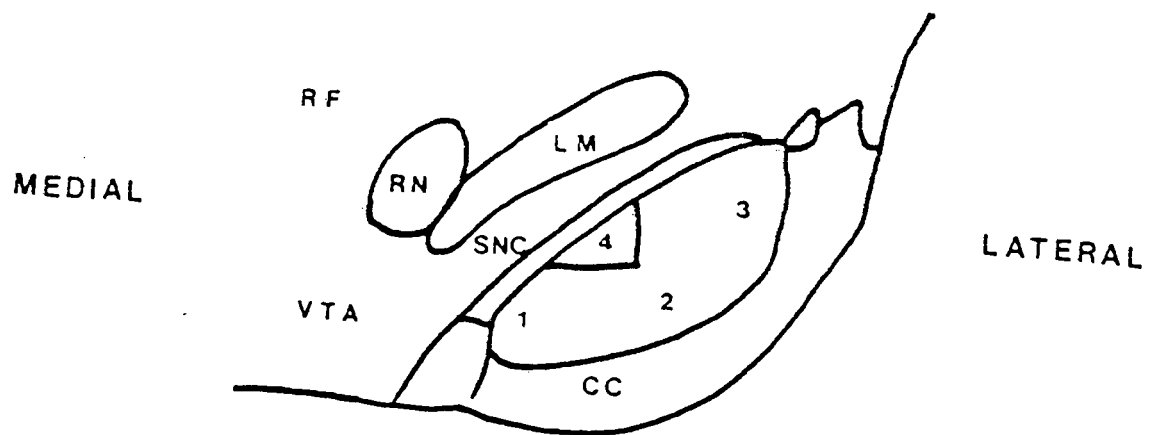
Both apomorphine and amphetamine result in ipsilateral circling. Bilateral kainic acid lesions of the SNR result in chronic stereotypy which is haloperidol resistant. Classically, the interpretation of these results has been that the kainic acid lesion blocks the expression of striatally derived circling at the nigral level (Olianas et al. 1978). However, the kainic acid induced circling was not susceptible to haloperidol; indeed, it was potentiated. The potentiation of circling observed may have been due to the inhibition of striatally derived stereotypy, allowing the expression of pure locomotor activity (Ljungberg and Ungerstedt 1978). These results suggested that the nigral DAergic system was not the only neuronal machine subserving circling behaviour. Olianas (1978) proposed the involvement of a neuronal system of SNR origin which played a role antagonistic to the NS DA system. The SN gamma-amino-butyric-acid (GABA) system was proposed to be inhibitory to this system.

1.5.2 NON-DOPAMINERGIC CIRCLING.

Evidence for non-DAergic circling of nigral derivation was obtained by the administration of GABA agonists and antagonists, microinjected via stereotaxically placed cannulae into discrete sites of the nigral complex. Muscimol, a GABA receptor agonist with a considerably longer duration of action than GABA (Naik et al. 1976), produces behavioural results reminiscent of striatal DA receptor activation and kainic acid administration when injected into the SNR (typically 5-50ng in 0.2-0.3 ul saline, Olanas 1978, Kilpatrick and Starr 1981). This muscimol effect depends on the site of nigral administration, suggesting multiple functional compartments within the boundaries of the SN. Several investigators reported contralateral circling in response to nigral muscimol (fig. 1.3B, Scheel-Kruger et al. 1977, Martin et al. 1978), which is paradoxical in the light of the classical view of striatonigral function subserving GABA mediated feedback inhibition of the NS system (Groves et al. 1975, Kim et al. 1971). If the SN pathway was solely involved in inhibiting the NS pathway, ipsilateral circling would have been expected. The systemic injection of muscimol was found to produce an increase in the firing rate of nigral DAergic cells (MacNeal et al. 1978, Walters and Lakoski 1978), and GABA or its agonists resulted in an increase in striatal DA release (Cheramy et al. 1978), and turnover (Biggio et al. 1977), when applied to the SNR, all of which suggests SNC activation. These results indicated that the SNR was not a homogeneous structure and that it could be divided

into functionally distinct zones with respect to its involvement in circling behaviour. Kilpatrick and Starr (1981) characterised the functional compartments of the SN with discrete nigral injections of muscimol (fig. 1.4). Muscimol (40ng in 0.2ul) injected into the SNC produced ipsilateral posturing of the head and trunk, or slow turning (7.2 turns per min. at 10-20 min), which later became contralateral, presumably as the drug diffused ventrally into the SNR. The ipsilateral circling was completely antagonised by haloperidol. Fast contralateral circling was obtained by injections into a centro-dorsal region of the SNR, 2167+-97 turns being completed in 90 minutes (mean +- standard deviation). This response was diminished to 641+-61 turns by ipsilateral 6-OHDA lesion of the MFB, and to 444+-64 turns with haloperidol (0.1mg/Kg) pretreatment. Circling elicited from the surrounding ventral and lateral SNR was also contralaterally directed, but of lower amplitude, typically 1400 turns, and was not affected by 6-OHDA or haloperidol. There is some controversy over the effect of the DA receptor blocker, haloperidol, on muscimol induced circling. Olanas (1978) demonstrated that haloperidol (0.2 mg/Kg) potentiated the circling to muscimol (5ng/0.3ul), from 16+-4 to 27+-4 turns per minute. This potentiation most likely stems from the use of a submaximal dose of muscimol, while Kilpatrick and Starr (1981) were overdriving the SNR with a dose an order of magnitude higher. Some investigators reported that haloperidol evoked a decrease in contralateral circling (500ng muscimol, Martin et al. 1978, while others reported no change (Oberlander et al. 1977). Thus, these results were further evidence for the co-existence and interaction

FIG 1.4. Coronal section through the substantia nigra complex indicating the functionally derived compartments. 4 indicates the centrodorsal zone^(of the SNR) hypersensitive to the ionophoresed drugs, 1,2,3 the ventro-lateral zone of lesser sensitivity. CC crus cerebri, LM medial lemniscus, RF mesencephalic reticular formation, RN red nucleus, SNC substantia nigra pars compacta, VTA ventral tegmental area. Redrawn from Kilpatrick and Starr (1981).



of both a DAergic and a GABAergic aetiology of circling. Additionally, the SNR could be divided into a centrodorsal and ventrolateral zone on the basis of circling intensity and DA dependence (fig. 1.4). The hypersensitive centrodorsal region (as a functionally derived compartment) extended rostro-caudally from A1.05 to A2.85 (Konig and Klippel 1963). These centrally placed somata have been shown to exhibit rostrocaudally extended dendrites. Ribak et al. (1980) reported that the SN projection terminates with greatest density in a centrodorsal core, which accounts for the higher amplitude of contraversive circling obtained when muscimol was injected into this region, but not for its capricious DA dependence. Centrodorsal muscimol infusion resulted in profound contralateral circling, which was markedly attenuated by haloperidol. Thus there were two processes enacted by the muscimol. Firstly, DA release in the ipsilateral striatum must have occurred, as DAergic circling is always away from the side of greatest DA release. This ipsilateral DA release implies a disinhibitory relationship between GABAergic neurones in the centrodorsal SN and the SNC DAergic neurones. Secondly, the residual haloperidol resistant contralateral circling points to a non-DA system involved in the behaviour. This non-DAergic system was exclusively activated by lateral or ventral muscimol infusion.

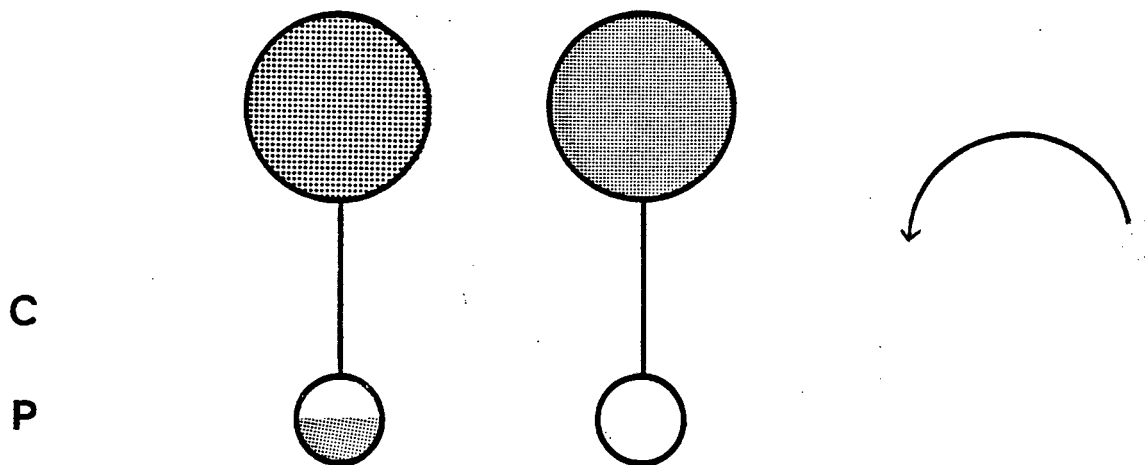
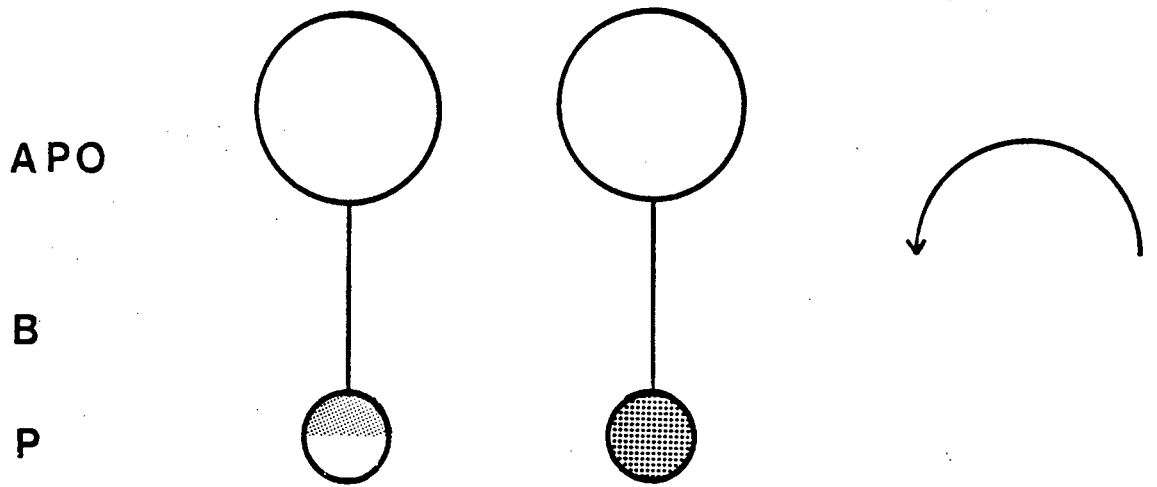
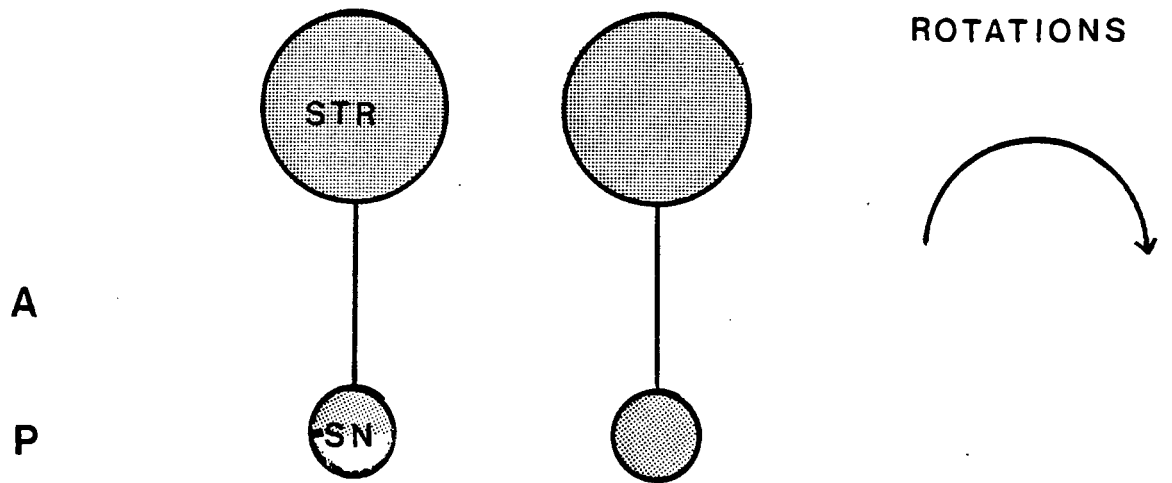
Nigral GABA receptor blockade with picrotoxin and bicuculline resulted in contralateral circling in response to high dose (200ng), and ipsilateral rotation to low dose (20ng, Olanas 1978) while others found only contralateral circling (Tarsy et al. 1975, Reaville et al. 1984, fig. 1.5A), or no effect at all (Martin et

FIG 1.5. The effects of intranigral picrotoxin. White shading indicates activated neural machine, grey shading, status quo. Arrows at right indicate rotation direction and intensity. Rotation intensity is proportional to the length of the arrow. STR, striatum, SN substantia nigra. P = picrotoxin, Apo = apomorphine.

A Picrotoxin infused within the substantia nigra pars reticulata produces contralateral circling by blocking the ipsilateral nigral GABA receptors.

B Apomorphine with concomitant intranigral picrotoxin produces ipsilateral circling by functional blockade of the apomorphine effect at the picrotoxin infused SN.

C Picrotoxin infused within the substantia nigra pars compacta produces contralateral circling due to blockade of the inhibitory effect of GABA on the NS projection.



al. 1978). Apomorphine co-administration produced ipsilateral circling (fig. 1.5B). Tarsey et al. (1975) and Olanas et al. (1978) aimed their microinjections at the dorsal SNR/SNC, while Reaville injected the drug more centrally within the SNR. Ipsilateral circling can be ascribed to inhibition of the SNC cells or to some non-nigral mechanism of circling, while contralateral circling occurred at high dose levels, or with infusion into the SNC. GABA receptor blockade of the SNC DAergic cells would release them from tonic GABA inhibition via the ongoing negative feedback of striatonigral GABA release. The blockade of GABA receptors would also impede striatonigral GABA mediated expression of circling via the thalamic and caudal SNR projections.

As described above, the SNR can be subdivided into compartments based on the behavioural results of nigral and striatal pharmacological manipulations. In addition, the SNR can be compartmentalised on anatomical grounds. The most dorsal SNR somata project to thalamic targets, while the more ventral cells form the caudal projections. There is also a persistence of glutamate decarboxylase immunoreactive nerve terminals in the central SN following striatonigral deafferentation, which suggests that there might be GABAergic local circuit neurones mediating SNR-SNC interactions (Ribak et al. 1980). Axon co-laterals from nigrothalamic projections may also form a substrate for local SNC control. There is sound electrophysiological evidence for opposite GABA effects on SNC (see earlier). SNC cell somata have been shown to possess GABA receptors (Kim et al. 1971) and ionophoretic

application of GABA to these cells produces a depression in their firing rates (Agajanian and Bunney 1974 cited in Grace and Bunney 1979), while paradoxical excitation has also been observed. Grace and Bunney (1979) presented evidence suggesting modulation of SNC cells by an inhibitory link from SNR. Simultaneous recording from SNC and SNR cells approximately 75um apart, showed there to be a sequentially alternating bursting pattern between them. Application of a noxious stimulus to the periphery induced inverse responses in SNC and SNR cells. Ionophoretic application of glutamate to the SNR cell resulted in its excitation with concomitant inhibition of the SNC cell, and vice versa with GABA application to the SNR cell. The SNR cells displayed 20 times greater sensitivity to GABA than did the SNC cells. Thus a functional link between SNR and SNC exists, with an inverse relationship between their excitation/inhibition status. The link may be a nigraly contained interneurone, or a reticulata-petal co-lateral, or even a multi-synaptic link.

There might even be no need to invoke an inhibitory nigro-nigral link to explain the electrophysiological results. Groves et al. (1975) pointed out that SNC cells can be inhibited by a process of lateral inhibition due to DA release from adjacent SNC cells activating DA autoreceptors. While this system has not been fully explored, it clearly could subserve local interactions within the SNC. According to this model, GABAergic inhibition of a SNC cell would release its neighbours from DA induced inhibition. Electrophysiological observations of GABA induced excitation in SNC may be a function of the spatial location of the recordant

cell. In the light of this it is interesting to note that Grace and Bunney used an oblique approach to SN which consistently staggered the SNR cells 75um ventral and 50um medial to the SNC cells. This means that they would have consistently recorded from SNC cells within the zone hypothetically disinhibited; released from lateral DAergic inhibition.

The striatonigral influence on SNC is thus more complex than the classical view of a feedback circuit inhibiting the nigrostriatal neurones. There is overwhelming evidence for this projection subserving striatal output via SNR. There are two routes whereby the SNR can express striatal function at spinal levels. The first is output via the thalamus, and the second, output via the midbrain. These will be dealt with sequentially in the next two sections.

1.5.3. BASAL GANGLIA OUTPUT VIA THALAMUS.

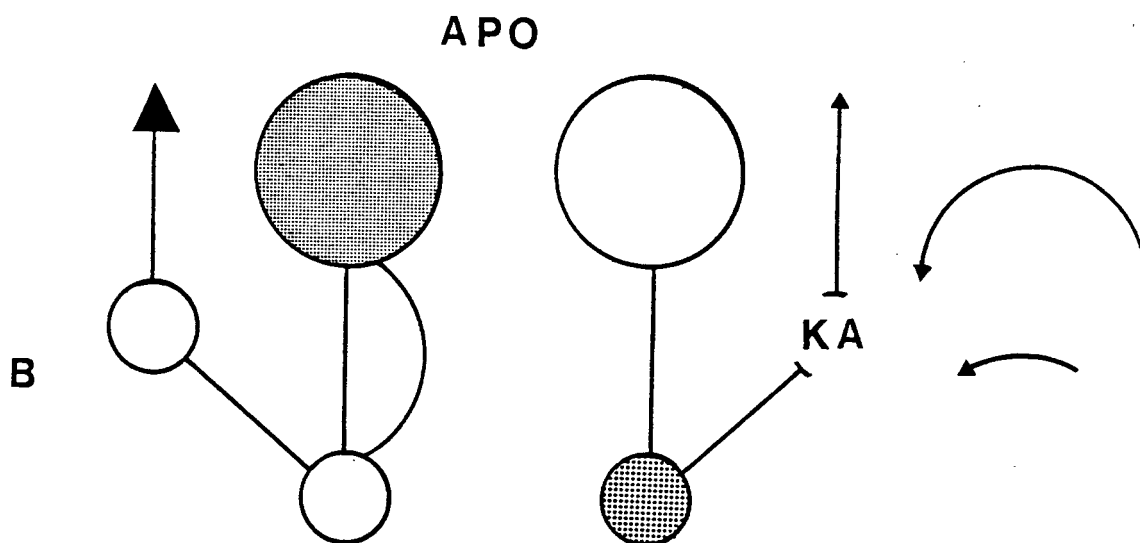
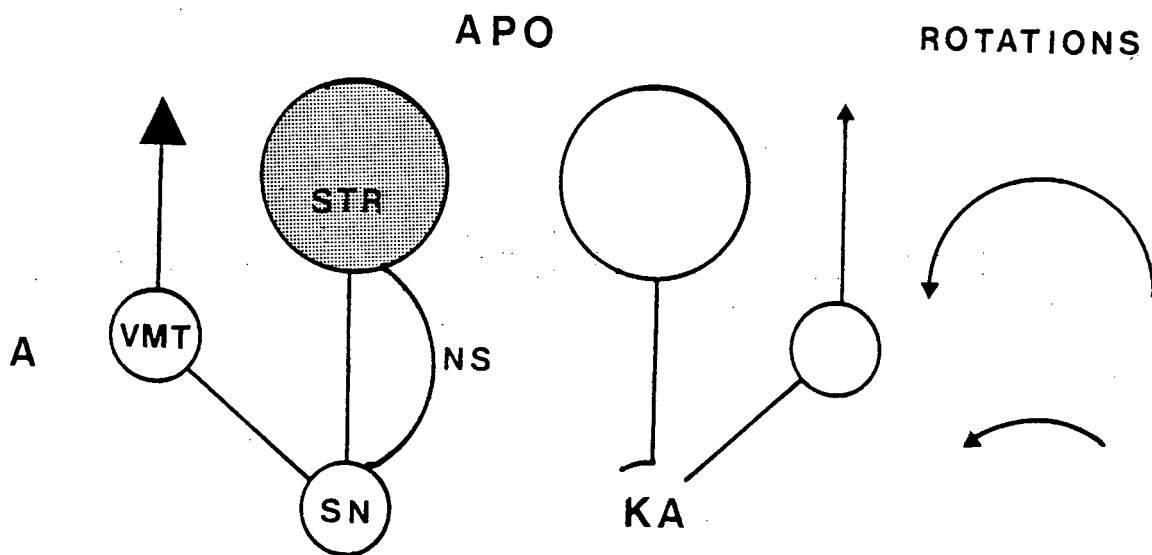
The basal ganglia are intimately involved with the thalamus. Both the internal and the external segments of the globus pallidus have projections to most of the thalamic nuclei, although the most prominent are the projections of the internal segment of the globus pallidus (GPi) to the ventro-anterior, ventro-lateral (VA-VL) complex, centrum medianum and lateral habenular nucleus. VA-VL thalamus projects to the supplementary motor area (Brodal 1982). SNR has a substantial non-DAergic projection to the ventromedial thalamus (VMT) which in turn projects profusely to the plexiform layer of the cortex (Herkenham 1976). Thus, thalamocortical projections are a means whereby striatal functioning might find expression as circling behaviour.

Lesions of the ipsilateral VMT were shown to produce a profound inhibition of the contralateral circling elicited by apomorphine (0.2 mg/Kg, i.p.) in the 6-OHDA lesioned rat, from 314 ± 53 to 70 ± 38 turns in 30 minutes (fig. 1.6B, Garcia-Munoz et al. 1983). Both kainic acid and electrolytic lesions were used in this study with essentially similar results. Lesions of the contralateral VMT had no effect on apomorphine induced behaviour, but bilateral lesions produced a decrease in contralateral circling from 202 ± 3 to 40 ± 4.7 turns per 30 minutes, behaviour essentially similar to ipsilateral VMT lesions. Leigh et al. (1983) showed that electrolytic lesions of the VMT ipsilateral to a 6-OHDA lesion of the NS system resulted in a decrease in contralateral circling

FIG 1.6. Schematic illustrating basal ganglia output via the thalamus. Drawn from the results of Garcia-Munoz (1977). The arrows at right indicate rotation direction and intensity. Rotation intensity is proportional to the length of the arrow. The right nigrostriatal system has been lesioned resulting in the right striatum becoming supersensitive to apomorphine. NS, nigrostriatal projection, STR, striatum, SN, substantia nigra. Apo = apomorphine.

A A kainic acid lesion of the right SN is performed. This results in an attenuation of circling from a subject with a prior 6-OHDA lesion of the right nigrostriatal system challenged with apomorphine. Dark shaded circle, normal striatal activity in response to systemic apomorphine. Clear circle, activity of striatum rendered supersensitive by 6-OHDA lesion of right nigrostriatal system in response to systemic apomorphine. Arrows at right indicate circling response before (1) and after (2) kainic acid lesion, in response to apomorphine.

B Identical to above except kainic acid lesion of ventromedial thalamus is performed. The attenuation of contralateral circling induced by this lesion indicates that the SNR-VMT is a route of expression for DAergic circling.



with apomorphine, but had no effect on amphetamine induced circling. If the VMT lesion was placed contralateral to the 6-OHDA lesion, apomorphine induced circling was unaffected but amphetamine induced circling was reduced. Bilateral VMT lesions did not affect circling induced by either drug. The contradiction of this result to that of Garcia-Munoz is not apparent from their publications. Soon after lesioning of the VMT with kainic acid, explosive locomotor activity and uncontrolled jumping was apparent. As kainic acid functions by inducing a state of neuronal hyperexcitability, this suggests that the SNR-VMT projection is involved in the activity component of circling behaviour. The results of these experiments are summarised in fig. 1.6.

These results suggest that VMT is important in the expression of circling behaviour. Note that lesion studies are interpreted as "blocking" the expression of the information generating the behaviour. Garcia-Munoz et al. (1983) make no mention of the compromise to the neural machine blocked. The amphetamine-apomorphine tracking of the relevant (lesioned) VMT is, however, compelling evidence that a striatal DA-dependent component of circling is expressed via a SNR-VMT projection and is associated with the processing of the VMT and its cortical projection sites.

1.5.4. BASAL GANGLIA OUTPUT VIA THE MIDBRAIN TECTAL AND TEGMENTAL REGIONS.

The region of mid-brain critical for circling behaviour has been termed the angular complex (AC), and includes the lateral PAG, dorsal MRF, and deep layers of the superior colliculus (SC) (Reaville et al. 1981). Included in this functionally relevant region would be any ascending, descending, or interconnecting fibre projections. The AC may be considered a focal point in a matrix of neural tissue concerned with postural and orientation mechanisms. Deducing from anatomical evidence, the AC might be a means whereby basal ganglia can influence the ongoing processing of the local circuitry, for convenience termed the midbrain locomotor area (MLA). This approach diverges somewhat from that encountered in the literature, where a demonstration of functional throughput for the basal ganglia to the bulbospinal mechanisms is sought (Reaville et al. 1979, Reaville et al. 1981). The existence of neuronal pathways from the SNR to nuclei of the midbrain concerned with locomotor activity and the description of routes whereby the spinal motor mechanisms can be influenced by midbrain nuclei receiving SNR projections, have fuelled this search for means of basal ganglia expression.

Nuclei of the midbrain locomotor area (MLA).

These are nuclei within the lower brainstem and mesencephalon. The lower brainstem is very much like the spinal cord. The spinal

motor neuronal cell groups continue into the lower brainstem. The medial spinal motoneuronal aggregate continues rostrally as a supraspinal nucleus continuous with the hypoglossal nucleus. This forms the most rostral of the spinal motoneuronal groups. The more rostral eye muscle nuclei, ie, abducens, trochlear and oculomotor nuclei, are linked to the hypoglossal nucleus. The reticular formation of the lower brainstem is similar architectonically to the spinal intermediate zone. It is characterised by cells with transversely orientated dendritic trees and medially located axial axons predominating. Caudal medulla oblongata contains the lateral tegmental fields (the bulk of the reticular formation). Medial tegmental fields comprise the paramedial reticular nuclei and interfascicular nuclei. More rostrally within the medulla oblongata, the medial tegmental fields form the reticular nuclei viz., nucleus gigantocellularis (nGC) and dorsal and ventral nuclei para-gigantocellularis. In the pons, the central pontine reticular nucleus forms the rostral extent of the medial tegmental field. In the rostral pons the medial tegmental field is joined by the nucleus subcoeruleus. The lateral tegmental field is replaced by the medial and lateral parabrachial areas (brachium conjunctivum). The bulbomotor nuclei comprise the facial nuclei, and also receive input from higher regions, the mesencephalic tegmentum and superior colliculus. The medial tegmental field nuclei control the eye nuclei. Relevant nuclei within the mesencephalon are the mesencephalic reticular formation, superior colliculus and vestibular nuclei.

The colliculospinal projection (tectospinal) originates in the

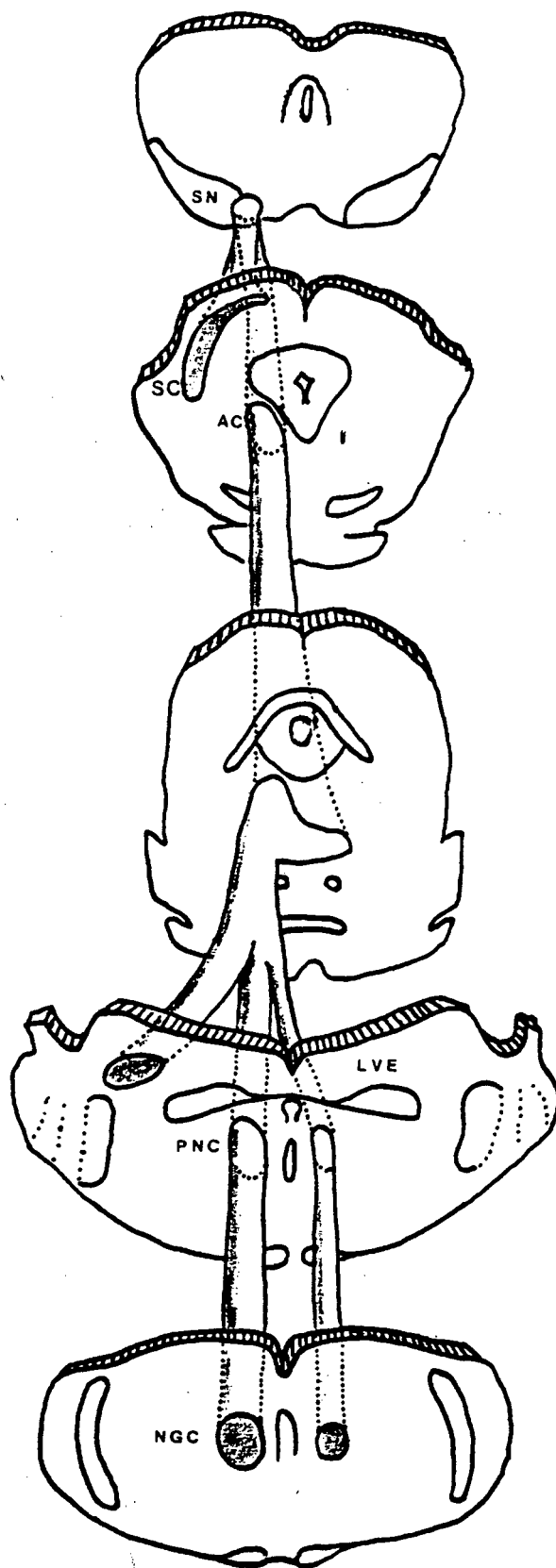
intermediate layer of the SC, suggesting a possible direct route for basal ganglia output via a SN-SC-spinal link up.

The PAG has also been demonstrated to originate a pathway to the spinal cord in the squirrel monkey (Mantyh 1983), which may be functionally relevant in circling.

There is also a projection to the AC from centro-dorsal SNR. A projection from AC to rostral nGC has been identified electrophysiologically as well as with retrograde and anterograde HRP. The anterograde HRP results suggested that the projection identified from AC to nGC extended rostrally as far as the centrodorsal SNR, although no combined electrophysiological and HRP confirmation of the functional nature of this projection exists. Electrophysiological evidence for a projection from SNR to nGC has been presented (Gonzales-Vegas 1981). The gradual diminution of the projection from AC to nGC levels suggests that it gives off fibres along its length to the surrounding MRF and PAG (fig. 1.7, Sefton 1985). The nigro-AC projection and the AC-nGC projection are likely to be continuations of the same projection, with a gradation of fibre distribution to AC and nGC regions.

It is still not clear how midbrain motor processing is expressed at the propriospinal levels. However, the reticulospinal tracts (RST) seem a likely candidate. This pathway has a definite functional role in the control of axial musculature. It originates in the four reticular nuclei and is compartmentalised into a

FIG 1.7. Schematic illustrating the anatomical substrate for expression of basal ganglia derived circling via the midbrain route. Fibres from SNR project to the intermediate layers of the SC and to the angular complex. The projection then courses to the nGC, giving off fibres to the surrounding neural substrate. Projections to the vestibular nuclei (LVE) exist and there is also crossing anterior to this. From the reticular nuclei, the reticulospinal projection may be a route affecting the bulbospinal motor mechanisms. SN, substantia nigra, AC, angular complex, PNC Pontine reticular nucleus LVE, lateral vestibular nucleus, NGC nucleus gigantocellularis.



lateral and medial tract (LRST and MRST). The reticular nuclei receive input from other regions associated with motor behaviour. The vestibular nuclei, a means whereby semicircular canal, otolith and labyrinthine influence is exerted on spinal levels, the cerebellum and the motor cortex, synapse on reticular cells in both excitatory and inhibitory modes. Termination of the RST within the spinal chord is non-somatotopic and extends over its entire length (Peterson and Wilson 1981). Thus the nGC might be a nexus for motor influence from the MLA, with expression at propriospinal levels occurring via the RST.

Conflicting results from lesioning and pharmacological manipulations of the nuclei comprising the AC have been reported in the literature, especially with respect to the involvement of this region in the expression of striatal DAergic circling. These results can, however, be explained on the basis of spatial location and extent of the lesion and/or drug infusion, and by taking into account the bias of data evaluation. What follows is a concise description and critical evaluation of the data pool.

Unilateral kainic acid or electrolytic lesions of the SC only evoked spontaneous behaviour when the lesions encroached on the dorsal mesencephalic reticular formation or involved the intermediate layer of the SC. Such unilateral lesions induced mild ipsilateral circling which decreased over 3 weeks, while bilateral damage induced hyperactivity and increased DA turnover in the n. Acc. (Reaville et al. 1979, Dichiaro et al. 1982, Dawburn et al. 1982). Apomorphine induced stereotypy was also reduced by

bilateral lesions. Lesions of the most lateral aspect of the SC were effective in producing spontaneous ipsilateral circling if they involved either the tecto-tegmental border, or the intermediate layer of the SC. The tecto-tegmental border is a region which receives projections from both SNR and circuits involved in otolith dependent postural reflexes (Garcia-Munoz et al. 1982). The intermediate layer of the SC, which receives the nigrocollicular projection and originates the colliculospinal projection, is most substantial in its lateral aspect. This possibly accounts for the greater responsivity of this region to lesioning and drug infusion. These animals were also found to be hypersensitive to environmental stimuli and nociceptive input, eg. tail pinch (Garcia-Munoz et al. 1982, Di Chiara et al. 1982), and exhibited deranged righting reflexes. In the presence of axon sparing kainic acid lesions of the deep layers of the SC ipsilateral to a 6-OHDA lesion of the MFB, apomorphine induced contralateral circling was much attenuated or directed ipsilaterally (DiChiara et al. 1982). The deep layers of the SC are, however, not the site of the nigro-SC projection, and are cytologically and physiologically similar to the reticular formation (Edwards 1980). The results of deep layer superior colliculus (DLSC) and dorsal MRF lesions can thus be interpreted as being of similar functional cause, ie. impairment of its processing function rather than attenuation of SNR expression, due to the preservation of axons by the kainic acid lesions. It is notable that unilateral lesioning of the tectospinal tract does not result in circling behaviour (Winterkorn 1980).

GABA receptor blockade of the DLSC/dorsal MRF with picrotoxin (12.5-50ng) or bicuculline resulted in contralaterally directed posture and slow circling (< 2rpm) when treated unilaterally. Bilateral injection resulted in stereotyped gnawing/biting. DA receptor blockade with haloperidol did not affect the asymmetry or stereotypy, thus implying a non-DAergic mechanism of action (Imperato and Di Chiara 1984). GABA blockade at more ventral sites and the lateral PAG resulted in running fits and seizures (explosive motor behaviour) and torsion about the axis. These behaviours suggest disinhibition of a locomotor pattern generator within the midbrain affecting posture autonomously. Hyper-excitability of the SC and dorsal MRF results in inhibition of spinal tonicity and the generation of stereotypy.

Intracollicular muscimol (2.5-25ng) induced ipsilateral circling with apomorphine (0.25mg/Kg) while only inducing spontaneous ipsilateral posture at high dose levels (200ng), which was compounded into circling with environmental stimulation. Bilateral muscimol (10-100ng) reduced apomorphine induced stereotypy, giving rise to a pure, compulsive, locomotor syndrome. Muscimol only induced circling behaviour when injected lateral to the PAG, in the DLSC/dorsal MRF junction. Muscimol thus stabilises the SC, resulting in an increase in ipsilateral postural tonicity and a suppression of apomorphine induced stereotypy. This suggests that the MLA exerts a tonic inhibitory effect on spinal motor reflexes, with SNR input modulating the MLA in the opposite direction, in terms of activity, to the DAergic system (Olianas 1978). Inhibition of the MLA by GABA receptor blockade produces an

increase in spinal toniciry.

Opposite effects in terms of activity as between striatal and MLA levels are logically consistent with isolateral postural bias. One might conclude that the postural bias of DA receptor stimulation is due to the operation of the GABAergic MLA, as these regions are directly linked to the spinal level, and can be autonomously activated by local neurotransmitter application. In the light of the induction of haloperidol resistant stereotypy by SC GABA receptor blockade, the stereotypy observed in response to DA receptor stimulation seems to be a function of the neural machinery caudal to the SN.

The lateral PAG, DLSC, and dorsal reticular formation are the regions of the midbrain most sensitive to lesioning and drug treatments. This is based on their efficacy in reducing apomorphine induced contralateral circling or inducing ipsi- or contralateral posture, circling, or stereotypy. This region of the midbrain was termed the angular complex (AC; Sinnamon and Miller 1980). A bilaterally AC lesioned animal with a prior 6-OHDA lesion of the NS system exhibited 155% increase in contralateral circling (fig. 1.8B, Starr and Summerhayes 1985). This seems to be due to the suppression of stereotypy, a result also obtainable with intra-collicular muscimol (Dawburn and Pycock 1982). Unilateral lesioning of the lateral edge of the PAG induced tight ipsilateral rotation following apomorphine (0.5mg/Kg), with concomitant depression of contralateral posture and grooming stereotypy. Some animals exhibited interdigitation of intense ipsi- and

FIG 1.8. Schematic illustrating manipulations of the angular complex on circling behaviour. White shading indicates activated neural substrate, light stippling, status quo, coarse stippling, reactive inhibition of neural substrate, black, inhibition of neural substrate. Horizontal lines show lesioned AC. Arrows at right indicate rotation direction and intensity. Rotation intensity proportional to the length of arrow. STR striatum, SN substantia nigra, AC angular complex. M = muscimol. Apo = apomorphine.

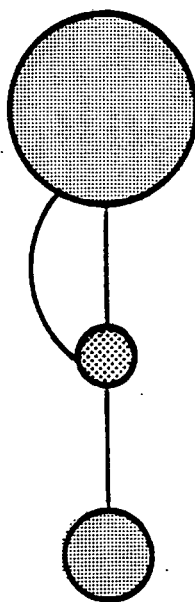
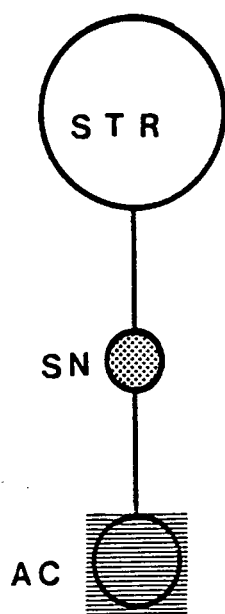
A. A unilateral lesion of the AC ipsilateral to a prior 6-OHDA lesion of the nigrostriatal system results in 1) interdigitated ipsi- and contralateral circling with apomorphine, with 2) contralateral circling ultimately persisting.

B Bilateral lesion of the AC results in 155% increase in apomorphine induced contralateral circling due to elimination of the stereotypy component of the behavioural syndrome.

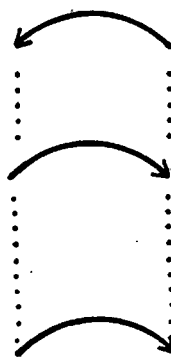
C Intra-AC muscimol results in ipsilateral circling due to activation of the inhibitory GABA system.

APO

A



ROTATIONS

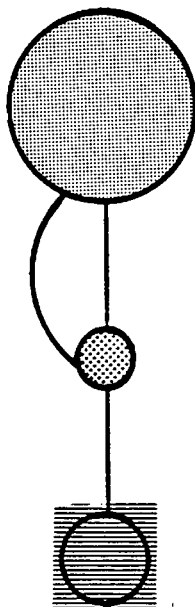
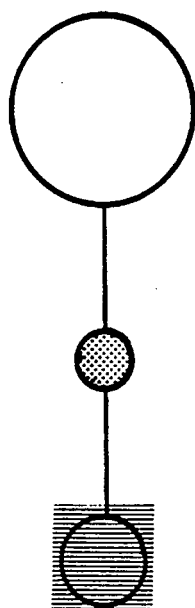


1

2

APO

B



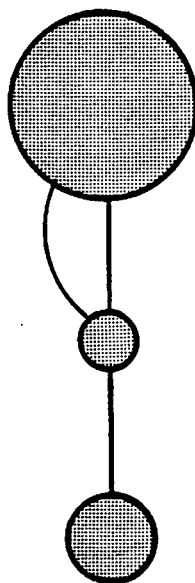
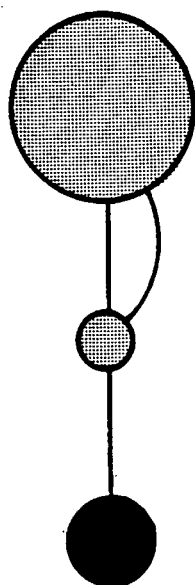
1



2

C

M



contralateral circling behaviour, suggesting two separate antagonistic mechanisms which competed for behavioural expression (fig. 1.8A. Starr and Summerhayes 1985). Interestingly, the same investigators obtained differential effects with apomorphine depending on the route of administration (Starr and Summerhayes 1982). Unilateral intracaudate apomorphine induced contralateral rotation was potentiated by an ipsilateral AC lesion, while the response to systemic apomorphine was attenuated, at least initially. Amphetamine did not evoke behaviour in conjunction with uni- or bilateral lesions. Drug infusion in the AC produced similar results (Starr and Summerhayes 1985, Reaville et al. 1984). Unilateral AC muscimol (100ng) produced ipsilateral circling at 9.4 turns per minute (fig. 1.8C). Unilateral lesion of the NS projection resulted in 4.4 turns per minute in the contralateral direction with muscimol infusion in the ipsilateral AC. Apomorphine (0.5mg/Kg) resulted in 16.3 turns per minute contralateral to the lesioned side, which was changed by concomitant ipsilateral AC muscimol to 8.0 turns per minute in wide circles to the ipsilateral side (fig. 1.9A). The AC derived muscimol-induced circling could thus override the striatally derived apomorphine-induced circling.

The contraversive circling induced by unilateral SNR muscimol was potentiated by 103+/-9% with intra-AC muscimol as well (fig. 1.9B, Starr and Summerhayes 1985), although others noted slow wide ipsilateral circling, not potentiation (Reaville et al. 1984). Intra-AC muscimol was better at suppressing apomorphine induced contralateral rotation than lesioning. Bypassing SN projection

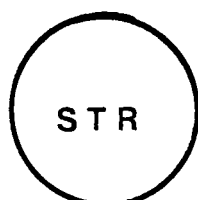
FIG 1.9. The effect of manipulations of the AC and SN on circling behaviour. White shading indicates activation of neural substrate, light stippling status quo, coarse stippling reactive inhibition of neural substrate, black inhibition of neural substrate. Arrows at right indicate direction and intensity of rotation. Rotation intensity is proportional to the length of the arrow. STR striatum, SN substantia nigra, AC angular complex. M = muscimol, Apo = apomorphine.

A Intra-AC muscimol infused ipsilateral to a prior 6-OHDA lesion of the nigrostriatal system results in ipsilateral circling with apomorphine. This is due to ipsilateral inhibition of the neural substrate by muscimol, while the contralateral side allows the full apomorphine effect to be produced.

B A potentiation of contralateral circling induced by intranigral muscimol results when the nigra is infused first.

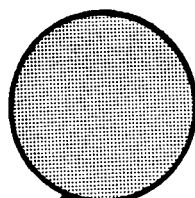
C A reduction of ipsilateral circling induced by intra-AC muscimol is obtained by a later intranigral infusion.

APO



SN

AC



ROTATIONS

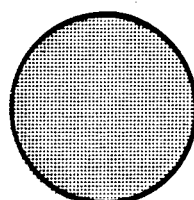


A

M

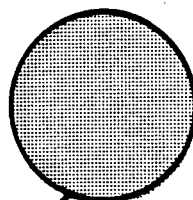
B

M



1

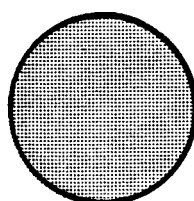
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1

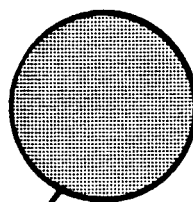


2



2

1



1



2

C

M

drive by muscimol infusion into the centrodorsal SNR resulted in contralateral circling which was potentiated or attenuated by intra-AC muscimol, depending on whether the AC infusion followed or preceded the SN infusion respectively (Reaville et al. 1984, Starr and Summerhayes 1985, fig. 1.9B,C). This, as with the interdigitated ipsi- and contralateral circling with AC lesion, suggests two oppositely directed drives. With lesioning, there would be competition between a contralateral drive induced by DA receptor stimulation affecting motor systems via ventromedial thalamus and the pyramidal tracts, and an ipsilateral drive from the disinhibited MLA expressed via the reticulospinal projections. In the lesioned animal the latter drive should predominate, being present continually, while the apomorphine drive would be superimposed over it.

GABA receptor blockade by intra-AC picrotoxin (100ng) resulted in contralaterally directed circling (7.6 ± 1.1 turns per minute) and stereotypy (Imperato and DiChiara 1981). These results suggest that the postural and gnawing components are derived from the AC compartment, while the catalepsy and locomotor component are of the VM thalamus compartment.

1.5.5 SYNOPSIS.

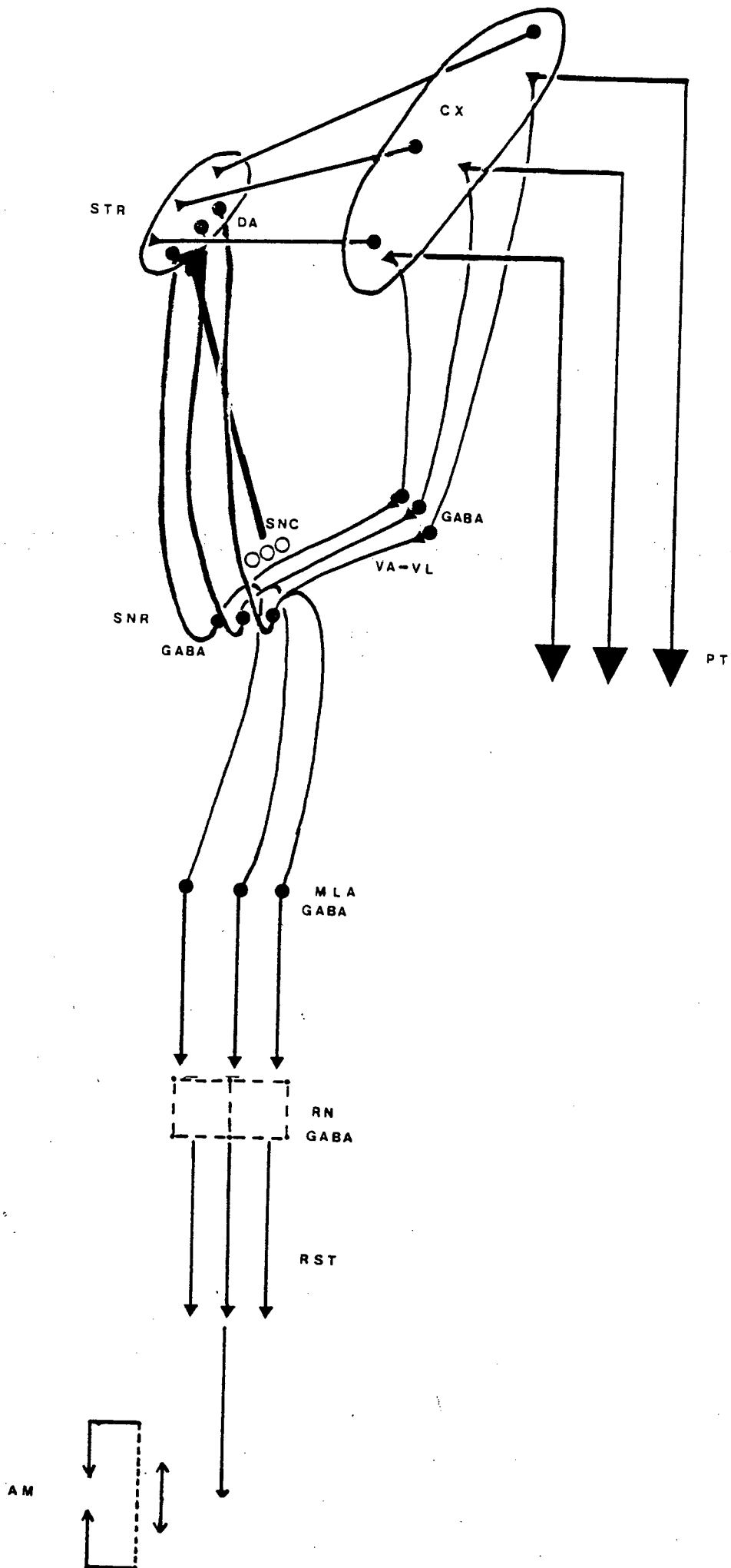
Experiments within the circling rat paradigm described above can be divided into lesioning, receptor agonism, and receptor antagonism. These manipulations have been performed at striatal (DAergic), nigral (GABAergic), and tectal/tegmental (GABAergic)

levels. The data presented have led to the evolution of a theory of rotating rats. The observation that established the prime criterion of this theory was that a unilaterally NS lesioned rat rotates ipsilaterally with amphetamine and contralaterally with apomorphine. As amphetamine acts presynaptically and apomorphine postsynaptically, the direction of rotation was consistently contralateral to the side of highest DAergic activity. The elegance of this system was that the direction of rotation could serve as an unambiguous indication of NS activity. The immediate relevance of these findings is their application for the screening of potential anti-Parkinsonian drugs for DAergic activity.

When research attention shifted to the output pathways of the striatum, the SN became the subject of multitudinous experimental manipulations, and a GABA paradox arose. The results of pharmacological treatments of the SNR are not consistent with the prime criterion of the rotating rat paradigm, namely that rotation is oppositely directed to the NS system of greatest activity. Intranigral muscimol and picrotoxin in an unlesioned animal results in contraversive circling. With apomorphine drive, however, ipsilateral circling is observable in both cases. This is interpreted as a blocking of striatal output, comparable to the effects of nigral kainic acid and electrolytic lesions blocking the transmission of striatally derived impulses to AC regions. The GABA paradox, then, is why should intranigral muscimol alone result in contralateral circling? If in the unlesioned state one SN is infused with muscimol, this should allow the other side to be more efficient, thus producing ipsilateral circling. The data

presented above suggest that there are two systems involved in circling behaviour which are defined in terms of their neurochemical, anatomical and behavioural identity (fig. 1.10). The DAergic system projects rostrally from the mesencephalic DAergic cells to the striatum and limbic associated nuclei. Striatal input from the entire cortex is probably associated with the DA action in the striatum. The GABA system is located within the SNR and MLA. This system has a direct interface with the spinal motor systems controlling the musculature. The two systems are interlinked by the striatonigral projection, which is the main striatal output pathway in the rat. The behavioural repertoire associated with the GABAergic system as determined by its persistence after haloperidol co-administration includes postural deviation, stereotypy, and locomotor activity. Behaviours induced by DA receptor effects alone are hyperactivity and catalepsy. Most of the behaviours ascribed to DAergic effects are inducible from the GABA system. The function of the DA system is inadequately reduced, but only indirectly involves that which describes circling in gross terms. Certain components like activity and sensory aspects eg. neglect, seem to pertain more specifically to the DAergic system.

FIG 1.10. Schematic of the wiring involved in the aetiology of circling. The SNC has an DAergic input to the striatum. The striatum also receives input from the cortex, and has output to the SNR (GABA). SNR can affect motor systems via VA-VLT (GABA) and motor cortex. The pyramidal tracts express this information. There is also a SNR output to the midbrain locomotor region (MLA, GABA) which is expressed via the RN and RST. The axial musculature is affected via this output pathway, while the pyramidal tract output affects musculature more discretely. Cx cortex, STR striatum, SN substantia nigra, VA-VLT ventroanterior, ventrolateral thalamus, MLA midbrain locomotor area, RN reticular nuclei, RST reticulospinal tract, PT pyramidal tracts, AM axial musculature.



1.6 THE NIGROSTRIATAL DOPAMINE SYSTEMS.

The NS projection is a crucial component in the description of the circling phenomenon, and the most striking pathology in Parkinson's disease is one of the DA producing cells of the SNC. Pharmacological DA depletion with reserpine (Goldstein et al. 1975), or bilateral striatal destruction, results in an akinetic Parkinsonian-like state. The similarity between the pathological lesion of the SN giant pars compacta cells in Parkinsonism, in the human subject, (Pycock 1983) and the artificial lesion of the same cellular group in the 6-OHDA rotating rat model, rally for the propriety of this model as an analogue for Parkinson's disease. The neuroanatomy of the NS system and the similarity of the 6-OHDA lesioned model to Parkinsonism will be examined in the next section.

The existence of a substantial NS projection has been suspected since the beginning of the century from observations of rapid cell atrophy in the substantia nigra following extensive destruction of the striatum, but attempts to demonstrate the connections directly by fibre degeneration methods succeeded only after the Fink-Heimer method in 1966. The first direct evidence of a NS fibre system originating from the DA cells of the ventral mesencephalon was reported by Anden et al. (1964) using the monoamine-histofluorescence technique. These cells were visualised as a large region of green fluorescence in the rostral mesencephalon, with a fibre tract continuous with caudate

fluorescence. The description of the structural organisation of the NS projection resulting from the application of the monoamine histofluorescence technique formed an important concept in the consideration of this system both within the circling rat paradigm and others.

Dahlstrom and Fuxe (1964) divided the collection of mesencephalic monoaminergic cells topographically into 3 groups: cells in the mesencephalic reticular formation (A8), in the SN area (A9) and in the basal-medial area mainly dorsal to the interpeduncular nucleus (A10) (fig. 1.11). The cells of group A8 were present dorsal to the lateral aspect of the medial lemniscus, extending into the dorso-medial regions of the mesencephalic reticular formation. More rostrally the red nucleus confines these cells to the lateral reticular formation and ventral red nucleus. The classification A9 was assigned to the cells of the SN complex. Most of these cells were present within the pars compacta of the SN, but several also within the pars reticulata and a few within the pars lateralis. Cell group A10 is the largest group in the mesencephalon situated caudally within the nucleus linearis, and more rostrally spreading laterally and ventrally into the ventral tegmental area (VTA). There is, however, no distinct anatomical separation between the A9, A10, and A8 cell groups; "...no distinct borderline was observed between zona compacta and area ventralis tegmenti and it was difficult to decide whether some of the green fluorescent cells belonged to the zona compacta or to the area ventralis tegmenti." (Dahlstrom and Fuxe 1964). The VTA is the region of small compacta type cells corresponding to the nucleus

FIG 1.11. Redrawn from Dahlstrom and Fuxe (1964). Coronal sections at various levels of rat brain indicating distribution of dopaminergic cell groups using histofluorescence techniques.

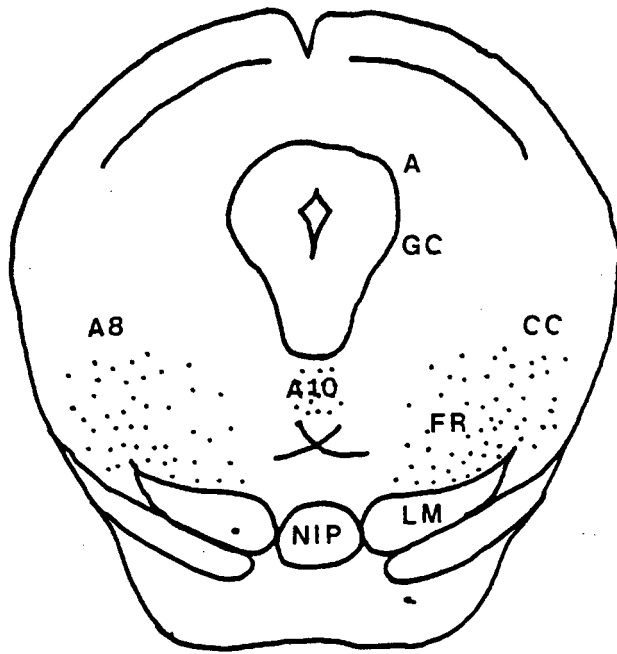
A. Transverse section through the mesencephalon at the level of the beginning of the nuc. interpeduncularis. The topography of groups A8 and A10 are illustrated. The catecholamine cell types are indicated with dots. AC Aqueductus cerebri, FR Formatio reticularis, GC Grisea centralis, LM Lemniscus medialis, CC Crus cerebri. NIP nucleus interpeduncularis.

B Transverse section through the mesencephalon at the level of the middle third of the nucleus interpeduncularis. The topography of groups A8 and A10 is illustrated. The catecholamine type cells are indicated with dots. NR Nuc. ruber SNC Substantia nigra, zona compacta; SNR Substantia nigra, zona reticulata. For other abbreviations see A.

C Transverse section through the mesencephalon at the level of the cranial third of the nucleus interpeduncularis. The topography of groups A9 and A10 is illustrated. The catecholamine type cells are indicated with dots. For other abbreviations see A-B

D Transverse section just rostral to the nuc. interpeduncularis. The topography of groups A9 and A10 is illustrated. The catecholamine type cells are indicated with dots. FRF Fasciculus retroflexus. For other abbreviations see A-C.

A



B

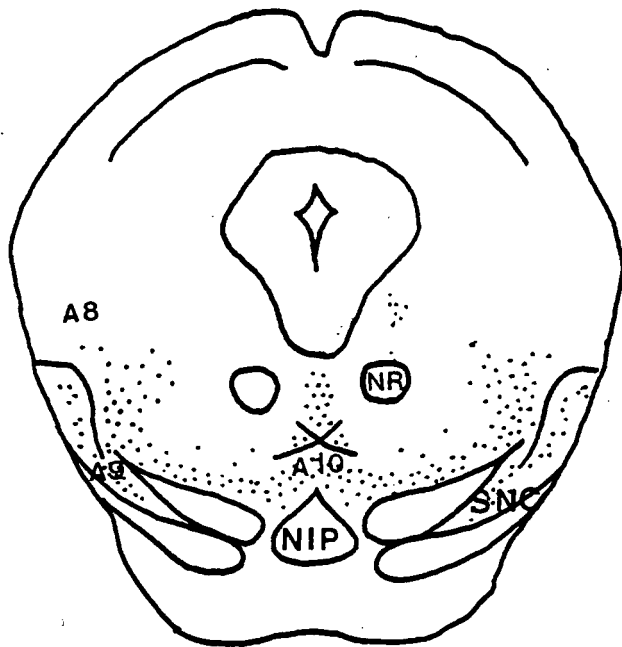
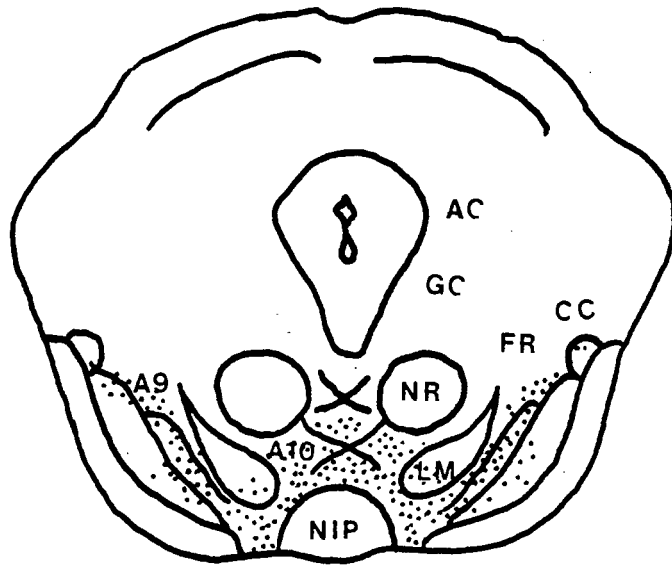
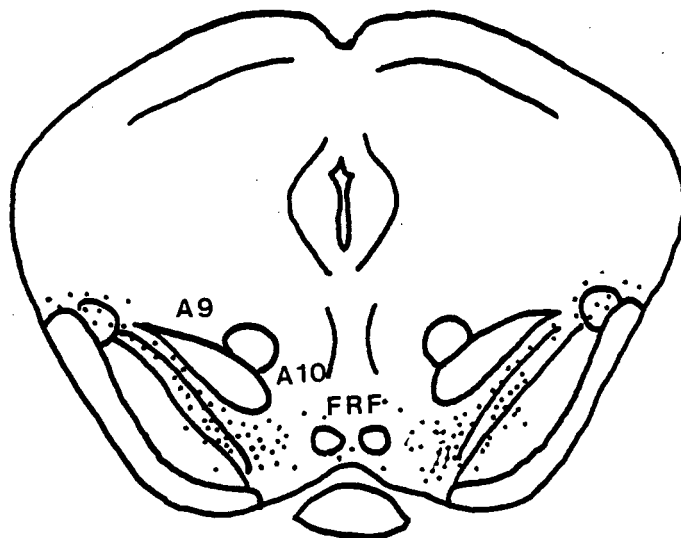


Fig. 1.11 continued.

C



D



paranigralis and nucleus of the mamillary body (Giguere 1984). Dahlstrom and Fuxe maintained that an arbitrary (though distinct in a functional sense) borderline could be drawn between the medial compacta and cells lateral to the interpeduncular nucleus, mainly on the grounds of the termination sites of the respective A9 and A10 projections. They maintained that the cells between medial SNC and the midline are not part of the nigro-neostriatal system, but rather project to the limbic system "...fluorescent cells within the area medial to the zona compacta and lateral to the nucleus interpeduncularis should preferably be referred to as group A10, since they probably are not part of the nigro-neostriatal system, but presumably of the limbic system." (Dahlstrom and Fuxe 1964). Laterally A9 was not clearly differentiated from the A8 cell group, and the cellular morphology of the two groups was identical. The cells of A9 mainly project rostrally via the crus cerebri and the capsula interna to the neostriatum (Anden et al. 1964). The rostral projection of A10 courses via the same route as the A9 projection. Dahlstrom and Fuxe are not very clear about the precise destinations of the projections their partitioning of the mesencephalic DA cell groups give rise to, although they use the criterion of terminal location to resolve the difficulty in assigning borders to the various cell groups. They regarded the A10 mesencephalic cell group as a meso-limbic projection and A9 as meso-striatal. Borderline cells were assigned to A9 or A10 on the basis of whether they projected to limbic or striatal targets.

Ungerstedt (1971) speaks more definitely on the nature of the

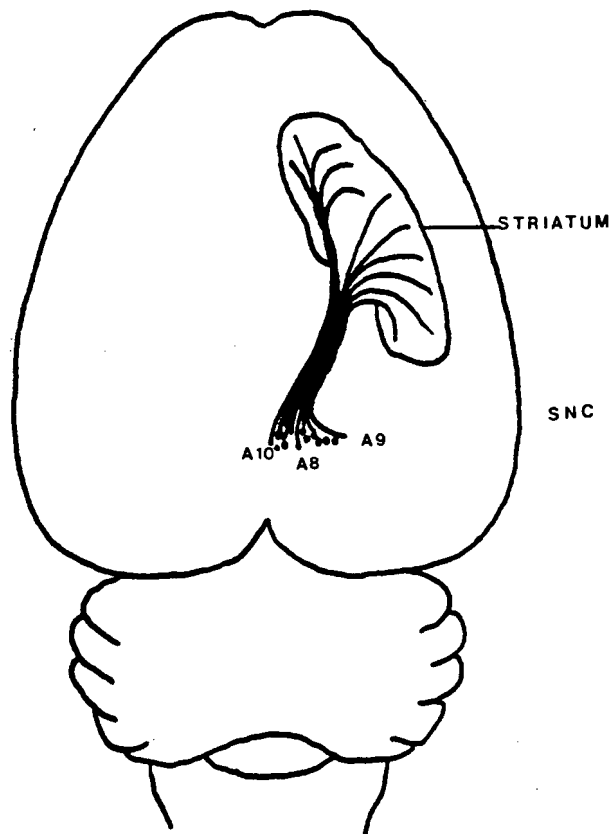
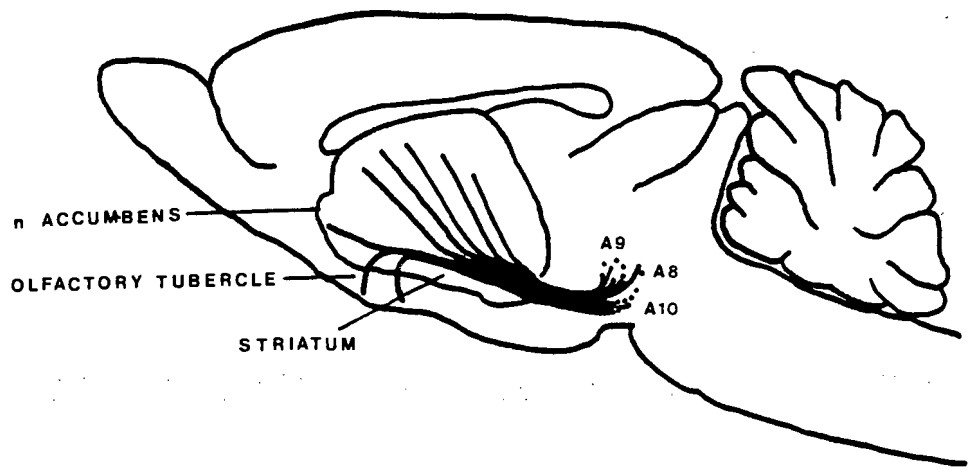
meso-diencephalic projections. Using the Dahlstrom and Fuxe classification he claims that the A9 DA cell group and its rostro-medial extension in the ventral tegmental area gives rise to a large projection that ascends in the lateral hypothalamus, enters the crus cerebri in the mid-hypothalamus, fans out in the globus pallidus, and enters the caudate nucleus. Lesions of the antero-ventral part of the A9 cell group, including the rostral VTA, produced a complete disappearance of terminals in the striatum, while caudolateral lesions affected only the cauda and lateral caput. The near complete striatal denervation produced by the antero-ventral lesion was probably due to its effect on projection tracts from more caudal structures. The A8 SNC cells course rostrally through the VTA, as do the axons from the A10 group dorsal to the interpeduncular neurones. Rostral antero-ventral lesion sites are commonly employed to induce circling. The meso-limbic DA system comprises the axons of DA cell somata dorsal to the nuc. interpeduncularis, corresponding to the cells seen in this study within the ventral tegmental decussation. These fibres ascend by a more medial route, never entering the crus cerebri, but proceed rostrally just dorsal to the medial forebrain bundle. At the level of the anterior commissure one branch enters the nucleus accumbens while the other turns ventro-laterally and enters the olfactory tubercle (Ungerstedt 1971).

The structure of the NS system as illuminated by the monoamine histofluorescence technique underwent a revision with the advent of autoradiography and the use of intra-axonally transported dyes

FIG 1.12. Redrawn from Ungerstedt 1971.

A. Sagittal projection of the ascending dopamine pathways in the rat.

B. Horizontal projection of the ascending dopamine pathway.

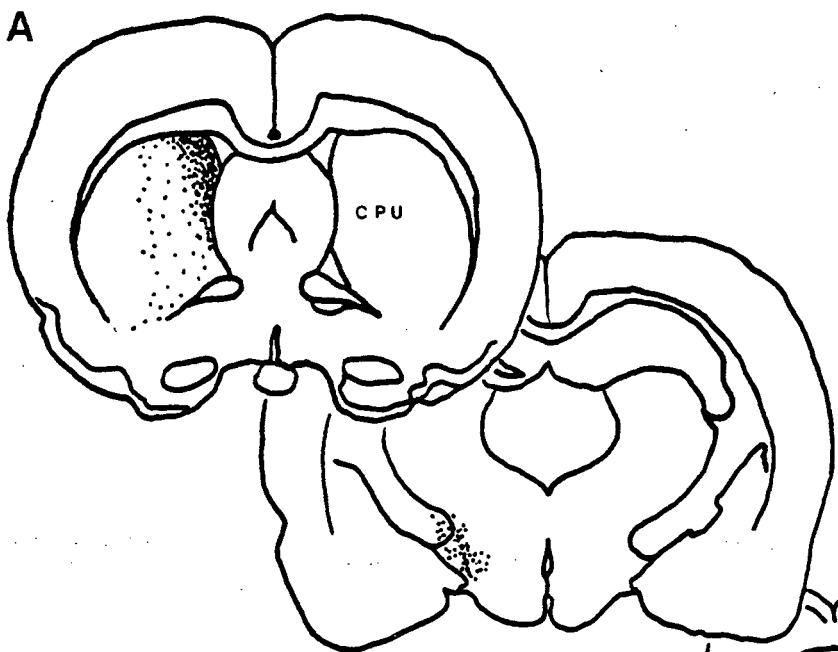


and enzymes. It is evident that the Dahlstrom and Fuxe description forms a subset of the contemporary description due to the resolution obtainable from the Falk-Hillarp method. Beckstead et al. (1979) used small quantities of tritiated leucine and proline injected into discrete sites of the ventral mesencephalon they considered important. The areas they investigated were the AVT, SNC, and SNR. Fibres incorporating tracer into them were visualised by the autoradiographic technique. Tracer injected into DA cells along the mediolateral plane from medial AVT to lateral SNC revealed a striatal labelling which maintained the topography of the injection zones. The most medial AVT deposition site labelled the striatum along its entire rostrocaudal length including the most anterior region, the n. Acc., and also the olfactory tubercle (fig. 1.13). The striatal region most densely labelled was the fundus (ventral) extending dorsally most medially, adjacent to the ventricle. Injections within the medial and middle SNC labelled the entire striatum, with the greatest density of labelled fibres in a dorsoventral column coursing steadily in concert with the injection site locations, ie. within the medial third and midway between the mediolateral striatal borders. The most anterior aspect of the striatum, n. Acc., was devoid of label. The most lateral injection of isotope resulted in the most lateral distribution of label in the striatum. There was no labelling of the anterior striatum, and the lateral labelling was distinctly ventral as well.

Beckstead et al. (1979) also made claims for an inversion of the dorsoventral topography of the NS projection. Due to its thinness

FIG 1.13. Redrawn from Beckstead et al. (1979). Coronal sections of rat brain showing the distribution of tritiated leucine and proline from discrete nigral deposition sites in medial (A), central (B), and lateral (C) substantia nigra pars compacta. As the injection sites proceed laterally, so the labelled fibres in the striatum follow. This indicated that the NS projection preserves mediolateral topography.

A



B

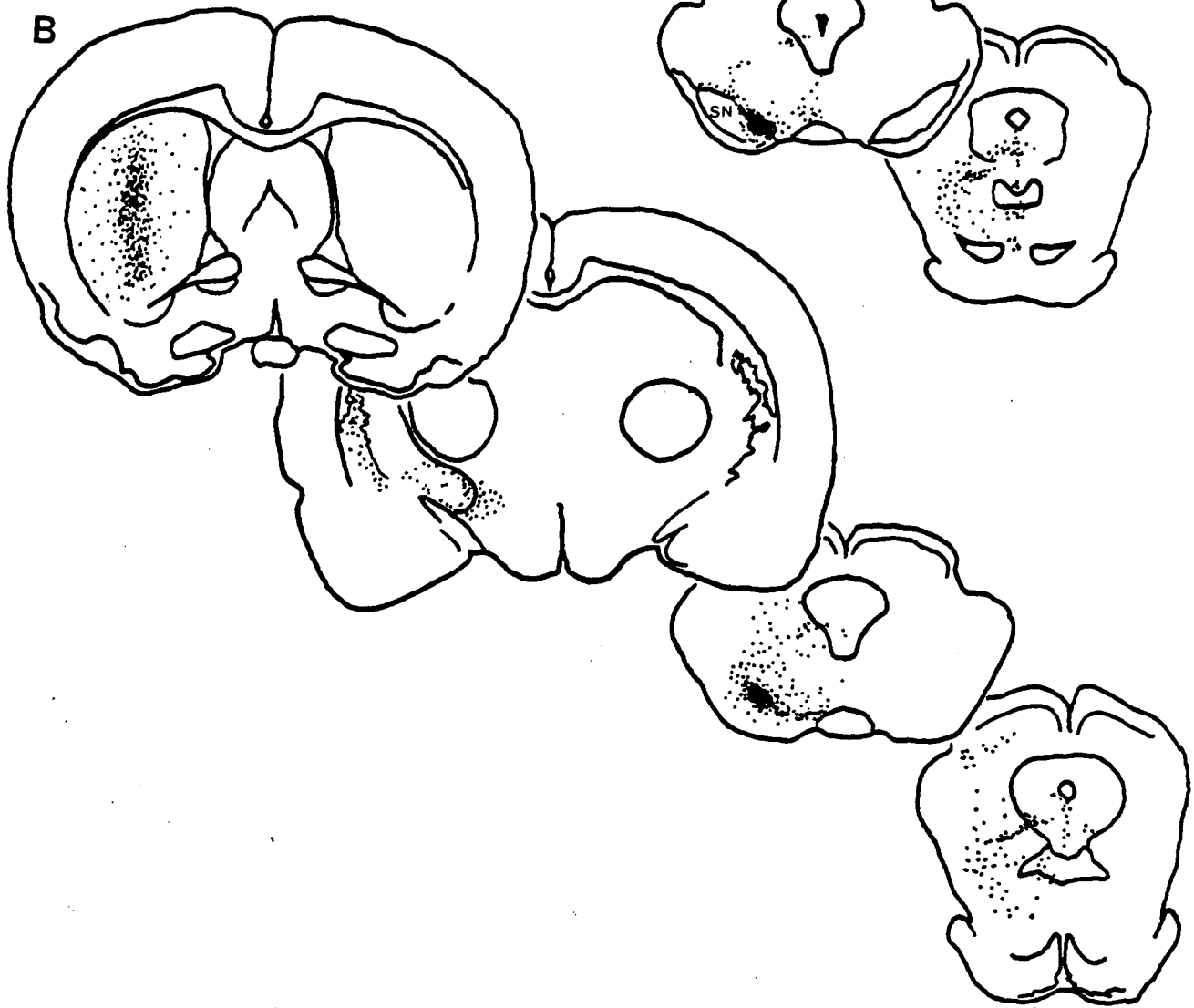
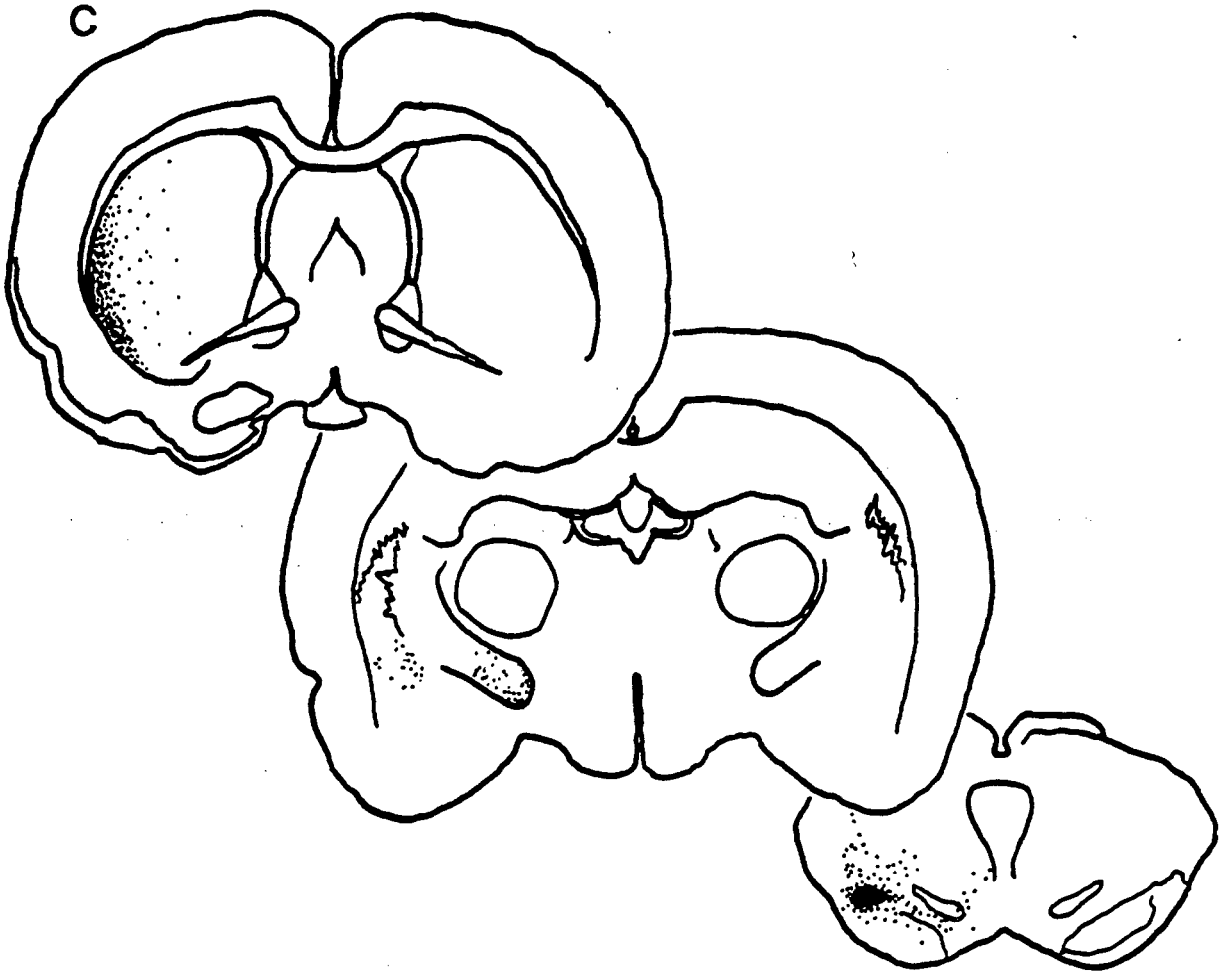


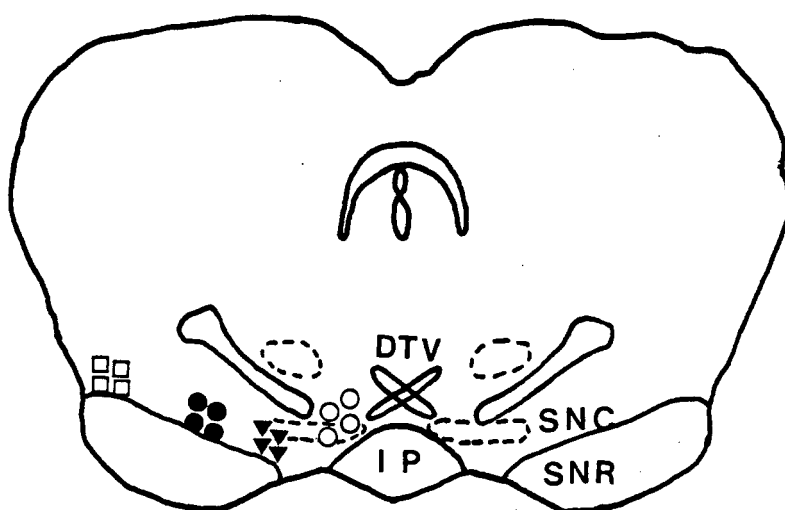
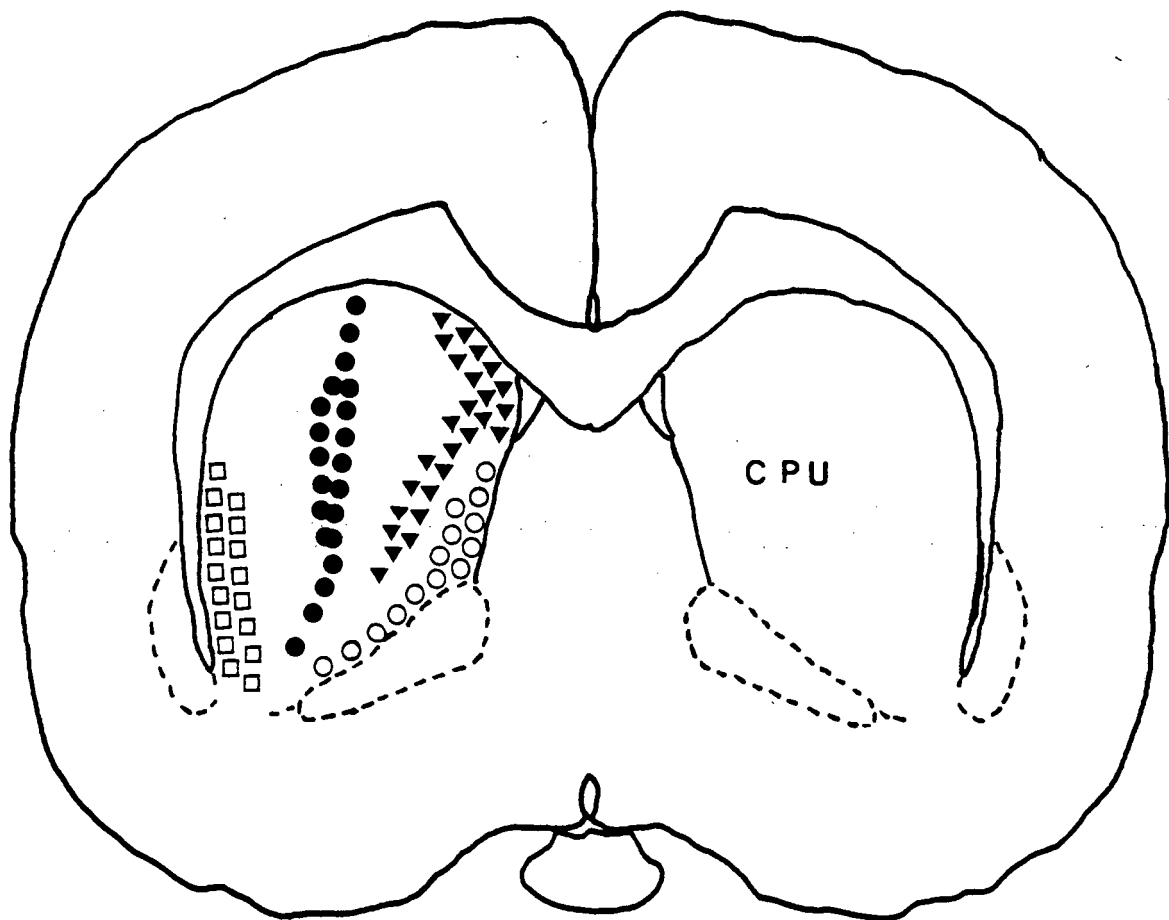
Fig. 1.13 continued.



the SNC alone could not be evaluated, but if the A8, A9, and A10 mesencephalic cell groups are considered as a continuum, the NS projection appears to invert its dorsoventral component (fig. 1.14). Similar assertions have been made by other investigators for the rat (Fallon and Moore 1978), and the monkey (Szabo 1979). However, the observation of dorsoventral inversion is explicable based on the folding of the striatum in a mediolateral way. Some of the most medial striatum appears to have folded ventrally and rostrally, while the most lateral striatum has folded ventrally and caudally. This is not a simple two dimensional folding; three dimensional structures both bend and move through themselves when folded. This accounts for the A8 and A10 projections reportedly projecting more ventrally (Most lateral or medial turns out to be most ventral). The striatum can thus be considered a folded-over-lozenge structure. This hypothesis is supported by the AP gradation of label observed by Beckstead et al. (1979) and Szabo (1980). If these observations are a function of folding due to spatial constraints then perhaps the n. Acc. is really a part of the most medial striatum.

The SN projection has indeed been shown to invert its dorsoventral co-ordinate, with certain functional implications. The most ventral regions of SNR would contain the terminals of the striatonigral projection from the dorso-lateral striatum; which in turn interfaces with the voluminous cortico-striate projection. The most dorsal regions of SNR would be innervated by SN projections from the ventro-medial striatum, which interfaces with the limbic nuclei. It is this dorsal termination of striatonigral

FIG 1.14. Composite schematic summarising the results of Beckstead et al. (1979). The mesencephalic depositions are depicted below with the resultant striatal distribution pattern shown above. There is a topographical organisation of the nigrostriatal pathway. Most lateral and medial labelling is also most ventral. This suggests a dorsoventral topography as well as a mediolateral one, though folding of the striatal tissue may also account for the observation of dorsoventral topography. Coronal sections from Pelligrino et al. (1979).



fibres which would perform the postulated GABA mediated feedback function on dendrites of the overlying SNC DAergic cells. The nigro-striato-nigral feedback loop is thus most likely to be of the dorsal SNR and medioventral striatum. Stimulation of DAergic and cholinergic receptors at caudate level modify multiunit spike activity in the ipsilateral SN. However, activity within the ventral aspect of the SN was increased while more dorsally activity was decreased (Pazo and Medina 1982). Tentatively it might be stated that the limbic striatal interface involves a feedback regulation between dorsal SN and ventromedial striatum, while the cortico-striate interface funnels into the output region of SNR.

NS projections from a specific nigral locus project to the entire length of the striatum. Beckstead et al. (1979) claims from these results that the designations A8, A9, and A10 have topographic value superimposed on a nigral cell continuum, each specifying a coherent nigral complex. The striatum has of the order of 20 times greater volume than the SN (Szabo 1980), and there is a considerable magnification (spatial expansion) of the NS projection from the nigral to striatal ends. As the striatum is functionally heterogeneous and the NS projection preserves topography, the nigral complex is likely to be of heterogeneous functional organisation as well.

The topography of the NS fibre tract is also of importance to the circling rat paradigm. The immunohistofluorescence technique revealed two definable rostral projection trajectories of the NS

system. The A9 cell group projecting via the lateral hypothalamus and crus cerebri, and the mesolimbic A10 projection ascending via a more medial route just dorsal to the MFB, never entering the crus cerebri. Most recently Giguere et al. (1984), using H3-leucine, described 3 main NS projections originating from different mesencephalic locations. The main bundle of neurones of the compacta type and corresponding to the (A9+A10), leaves the SN from its dorso-medial lip. They proceed rostrally through the ventral tegmental area of Tsai, via the lateral hypothalamus and sub-thalamic nucleus to the internal capsule. At the level of the anterior limb of the internal capsule they undergo an arborisation before ending in the caudate-putamen. A claim is once again made for a reflection of the dorso-ventral component of this projection with termination being in the DA islands (clusters of striatal cell concentration) of the striatum. Smaller neurones of the compacta type located within the medial SN and adjacent VTA (A10) project rostrally along the same trajectory as the main bundle of NS fibres but terminate predominantly in the ventral striatum and the nucleus accumbens. A third NS fibre tract originates in the lateral aspect of the rostral two thirds of the SN, the SN pars dorsalis (SND). This name is often used to include the retrorubal nucleus and A8 (Szabo 1980).

Beckstead et al. (1979) found that the NS fibres projected rostrally according to a mediolateral pattern which followed logically from the focus of the injection site. Most medial injections located within the VTA illuminated fibres within the most medial MFB. Injections located within the lateral SNC or SND

labelled fibres within the lateral MFB and medial cerebral peduncle. The most lateral fibres divert laterally to innervate the caudal striatum, while the most medial fibres continue rostrally to innervate the more medial and rostral regions of the striatum, including its rostral extent, the n. Acc. (Szabo 1980, Beckstead et al. 1979).

This anatomical distinction has been used to differentially deplete the DA content of the limbic and non-limbic striatum. Rostral LHA 6-OHDA lesions spare the striatal innervation while depleting the limbic n. Acc. and olfactory tubercle, thus producing a lesion specific to the mesolimbic system (Reaville et al. 1979).

Dahlstrom and Fuxe's classification of A10 as being the meso-limbic DAergic projection has therefore to be revised. The confusion surrounding the Dahlstrom and Fuxe schema arose from the categorisation of the mesencephalic DA cells in terms of their spatial distribution within the mesencephalon and their cellular morphology on the one hand, and their functional termination site on the other. This definition arose due to the resolving ability of the immunohistofluorescence technique and the intent of the investigators to compartmentalise the NS projection in a functionally useful way. A10 was initially defined as the mesencephalic site of origin of the meso-limbic projection, and described the cells dorsal to the interpeduncular nucleus extending laterally as far as the medial aspect of the SNC (fig. 1.11). Recent evidence has shown that while this region does

innervate the classically defined limbic n. Acc. and olfactory tubercle, it also projects to the ventral quarter of the entire length of the striatum (a region of which n. Acc. forms the anterior part). This striatal innervation extends dorso-medially as well, where it overlaps with the NS projection of the medial SNC (Dahlstrom and Fuxe's A9). The n. Acc. is defined more by its poverty of innervation from the SNC (A9) than by its sole agency of A10. That a medial or lateral-hypothalamic trajectory was invoked by Ungerstedt (1971) when addressing the mesolimbic A10 or A9 projections respectively, is understandable as a subset of a mediolateral continuum of topographic spread within the fibre tract due to the coarse resolution of the immunohistofluorescence technique. The Dahlstrom and Fuxe A10 label can be viewed as a lumping together of that part of the topographic spread mainly concerning the limbic n. Acc., olfactory tubercle and cortex. The striatum has also to be redefined into a ventromedial zone (the limbic striatum Kelly et al. 1982), projected on by limbic and limbic associated regions and the "feedback " loop from the nigra, and a dorso-lateral zone, projected upon mainly by motor cortex.

The anatomical heterogeneity of the NS projection probably has functional importance in circling behaviour. Classically, large lesions of the ventral mesencephalon (Ungerstedt 1971) or medial forebrain bundle have been employed to induce ipsilateral rotation. The lesion location most often encountered in the literature is just anterior to the SNC, ie. at the origin of the MFB. These lesions disrupt the DAergic innervation of a number of structures, not merely the striatum. But more discrete approaches

suggest that the role of the SNC in circling behaviour may be less significant than previously believed (Hodge and Butcher 1977). Lesions of the pars compacta of the SN (A9) reduce the DA levels of the striatum to 40% whereas MFB lesions reduce DA to subdetectable levels. Robust circling is the result of MFB lesions, while only mild ipsilateral circling behaviour results from restricted SNC ablation (Hodge and Butcher 1978). Thus it seems that the limbic nuclei and their afferent projections from A10, and the striatal innervation from SNC have functionally distinct effects on circling. The limbic nuclei and A10 are implicated in the motor activity component of circling (Kelly and Moore 1976), while the motor asymmetry component is associated with the striatum and the SNC (Pycock et al. 1978). Neurones of the nucleus accumbens exert an inhibitory effect on activity, as amphetamine administration produces an increase in n. Acc. DA release, depression of neuronal firing rates, and an increase in locomotor activity (Jackson and Kelly 1983). n. Acc. ablation induces a hyperactivity which is not potentiated by amphetamine. This functional compartmentalisation extends to the afferent projections of the limbic and non-limbic striatum. Lesions specifically of the A10 cell group induce hyperactivity without lateralisation (Galey et al. 1977).

1.7 THE PHENOMENON OF FUNCTIONAL INTERDEPENDENCE BETWEEN THE NIGROSTRIATAL SYSTEMS

The NS systems have always been presumed to be exclusively ipsilateral projections as they were first described in detail by the pioneering work of Dahlstrom and Fuxe (1964) using immunohistochemical fluorescence techniques. The interdependence of the NS systems was alluded to by Nieoullon et al. (1978), observing bilateral regulation of H3DA release in the caudate nuclei of cats implanted with push-pull cannulae. For example, H3DA release was markedly increased in the contralateral caudate nucleus after unilateral electrocoagulation of the ipsilateral SN, while decreasing H3DA release in the ipsilateral caudate, as expected. This response occurred immediately after the lesion, reaching 75% depletion ipsilaterally 90 minutes after lesioning. The contralateral increase in H3DA release lasted for 45 minutes and peaked after 30 minutes, 275% above control values. In order to circumvent the problem of non-specificity inherent in electrolytic lesions, various pharmacological means were attempted to inhibit the firing of one NS system. Perfusion of the ipsilateral SN with H3DA ($10^{-7}M$), benztropine ($10^{-6}M$), or amphetamine ($10^{-6}M$), induced results similar to NS lesioning. These pharmacological treatments all have the effect of increasing [DA] within the SN. It is hypothesised that this would result in the inhibition of the NS system via stimulation of DA autoreceptors on the SNC somata and dendrites (Agajanian and Bunney 1973, Bjorklund and Lindvall 1975). Unilateral interruption

of signals in the SN therefore seemed to influence the activity of NS neurones on the contralateral side. In addition to the lesion and pharmacologically induced changes in [H3DA], the spontaneous fluctuations in the [H3DA] were observed. They were in opposite directions as between left and right striata (Nieoullon et al. 1977).

The depressing effect of NS lesioning on striatal DA release is a short term phenomenon, and the medium term degeneration release is not relevant here as it is independent of neuronal activity. When viewed over the long term, Chandu-Lall et al. (1970) found that unilateral extirpation lesions of the caudate produced depletion of both the ipsi- and contralateral SN by 74% of control values 2-3 weeks after lesion. This is preliminary evidence of metabotropic compensation ordered to reduce the imbalance between the two sides.

That neuronal activity is a sine qua non in the interdependence phenomenon is the evidence of Hull et al. (1974) using single cell recording techniques in the cat and monkey. The activity of cells in the left caudate increased following lesioning of the ipsilateral NS projection. A simultaneous reduction of the firing rates of cells in the contralateral caudate was observed, and an increase in the firing of contralateral SN cells.

In addition to reciprocal NS [DA] control, it has been demonstrated that similar reciprocal nigral [GABA] interdependence exists in response to caudate nucleus activation by electrical

stimulation or local acetylcholine microinjection.

The studies cited above suggest that the NS DA systems are in reality functionally linked. One of the questions raised by this deduction is how messages originating in the SN on one side reach the contralateral DA neurones. This led to the notion that anatomical interconnection between the NS systems was likely.

1.8 NEURAL SUBSTRATES FOR INTERHEMISPHERIC COMMUNICATION OF THE NIGROSTRIATAL SYSTEMS

Anatomical interconnection between identical brain structures within left and right hemispheres is not uncommon. The corpus callosum (CC) is one of the most striking fibre tracts within the CNS of neo-amphibians, connecting related structures in the di- and telencephalic hemispheres, being primarily composed of crossed cortical association fibres. In addition to the CC there are other interhemispheric fibre tracts which are primarily responsible for interconnection of mesencephalic structures, and may mediate interhemispheric communication. These include the anterior commissure, posterior commissure, thalamic commissure, dorsal tegmental decussation (DTD), ventral tegmental decussation (DTV), and the supra-mamillary decussation (SMD). These are specific crossed projections, usually composed of a fibre population of various types. Homologous brain structures are not the only regions with interhemispheric connections, indeed projection tracts between various nuclei have been shown to be crossed. There are thus a number of decussating fibre projections and

multisynaptic circuits which may contribute to interhemispheric NS communication.

Summary of projections possibly subserving interhemispheric NS function.

a) Corpus callosum/striatonigral circuit

Reciprocal DA effects within the left and right NS systems were blocked by transection of the anterior corpus callosum (Nioullon et al. 1978). This suggests that cortical association fibres are involved in the interhemispheric communication between the NS systems.

b) Lateral hypothalamus/SN projection

A crossed pathway from the caudal lateral hypothalamus (LHA) to the contralateral SNR has been demonstrated by WGA-HRP tracing in both retrograde and anterograde directions. Reciprocal tracing from LHA deposition sites revealed VTA and SNC innervation of the ipsilateral LHA. This labelling was considered most likely to be due to label uptake by ruptured fibres due to cannula damage of the NS pathway, though the possibility of boutons en passant from the NS fibres synapsing with the crossed LHA-nigral projection may provide a substrate for nigro-nigral projection (Gerfen et al. 1982).

c) Pedunculopontine (PPn) crossing.

There is a caudally directed projection from the SNR to the PPn in the rat which is reciprocated bilaterally. The PPn also projects bilaterally to the subthalamic nucleus (Gerfen et al. 1982).

d) Cortex and direct projection influence.

Direct projections from the motor cortices to the striata bilaterally and from other cortical areas unilaterally, as well as a direct corticonigral projection have been described (Kemp and Powell 1970, Rinvik 1966). The crossed component of these pathways would have been transected by the corpus callosum lesions in the study of Nioullon et al. (1978), which implies that these direct, glutamatergic, corticostriatal and nigral pathways may mediate reciprocal NS control.

e) Crossed nigrothalamic projections.

The ipsilateral SN innervates the ipsilateral parafascicular nucleus and the ventromedial thalamic nucleus bilaterally. These thalamic motor nuclei have been implicated in reciprocal NS system control (Cheramy et al. 1981). The VMT nigrothalamic projection has been suggested by Pritzel and Huston (1980) to undergo enhancement after unilateral SN lesion. The possibility of the contralateral, intact SN, innervating the denervated ventromedial thalamus as a compensatory mechanism is thus raised.

f) Crossed nigrostriatal projection

A NS pathway from the SNC to the striatum of the opposite hemisphere has been described in rats (Fass and Butcher 1981). This pathway is a likely candidate for interhemispheric DA control, due to coincidence of the crossing and the DAergic nature of the pathway (Fallon et al. 1983). Other crossed pathways are GABAergic (nigrothalamic (DiChiara et al. 1979), and nigro-pedunculopontine), glutamatergic (cortico-striatal and cortico-nigral (Nieoulon et al. 1978)), or of unknown neurotransmitter specificity, but certainly non-DAergic.

1.9 THE CROSSED MESOSTRIATAL PROJECTION.

The use of neuroanatomical tract tracing procedures has primarily been responsible for the identification of minor fibre pathways in the brain, like the crossed mesostriatal pathway (Royce 1978). Horseradish peroxidase (HRP) was deposited in the caudate nucleus of the cat. There were labelled cells in the ventral mesencephalon ipsilateral to the HRP injection after one or two days. There was significant labelling of the SNC, the ipsilateral VTA, and surprisingly, a few cells in the contralateral VTA and SNC. Fass and Butcher (1981) presented evidence for a similar sparse crossed NS projection in the rat using the fluorescent dye Evans blue (EB) as a retrogradely transported label. The dye infusion was typically restricted to the rostral head of the caudate, and the retrograde labelling of the ventral mesencephalon revealed EB

fluorescence within the ipsilateral SNC, ventromedial mesencephalic tegmentum and a few labelled somata within the SNR, considered to be displaced SNC cells. Although no cell counts were done, the density of labelled somata was found to vary from dense labelling in the rostral SNC to lighter labelling in the caudal pole of the nucleus, and the converse in SNR. Several (14 to 2 per brain) EB labelled somata were found in the contralateral SN and VTA. If the infusion site did not impinge on the striatum no ipsi- or contralateral labelling of mesencephalic cell somata was apparent. The pattern of labelling obtained with an optimal infusion restricted to the rostral head of the caudate was not altered by transection of the corpus callosum. This implied that the crossed projection decussated somewhere other than the corpus callosum.

Thus, there was a contralateral contribution to the predominantly ipsilateral NS projection, although its level of decussation and functional importance was still uncertain. The neurochemistry of the crossed projection was suggested to be largely catecholaminergic (Fallon et al. 1983). Cell counts of the ipsilateral and crossed mesostriatal pathways suggested that the crossed projection was 1-2% of the ipsilateral projection, and that 93% of the contralaterally labelled cells were DA containing (Fallon et al. 1983). In a study using a double labelling technique, Altar et al. (1983) found that the contralateral projection was 1% of the ipsilateral projection, and that no cells projected to both caudate nuclei. Lesioning the ipsilateral mesostriatal projection at the level of the lateral hypothalamus

with 6-OHDA in neonatal rats produced a marked striatal DA depletion, of the order of 85% 6-9 days post lesion and 68% 20-42 days post lesion. The lesion reduced both ipsilateral and crossed cells labelled with tracer by an equivalent percentage. The axons of the crossed projection must therefore already have been incorporated into the ascending meso-diencephalic tracts, which follows two major trajectories; the "A10" projection via a medial projection just dorsal to the medial forebrain bundle, and the "A9" projection which ascends in the lateral hypothalamus. Intuitively, it seems unlikely that the 6-OHDA injection of 2ul in a neonatal rat would have been contained within the lateral hypothalamus. The neurotoxin must surely have spread from its original lateral hypothalamic target site to the more medial DA projections. On the basis of this data the NS trajectory incorporating the crossed projection cannot be specified. However, the level of decussation must have been between a coronal plane through the ventral mesencephalon and one through the lateral hypothalamus.

The Pritzel, Huston and Sarter experiment

Pritzel et al. (1983) reported that unilateral surgical ablation of the telencephalon resulted in spontaneous circling behaviour which "attenuated markedly" within one week (numbers of rotations not specified). HRP injection into the ipsilateral thalamus revealed a greatly increased number of labelled somata in the contralateral mesencephalon. Unilateral lesions of the mesencephalon were performed as well (Pritzel 1983), using 6-OHDA

or kainic acid according to the atlas of Hurt et al. (1971; AP=2.5, L=1.5, D=2.5). The 6-OHDA dose was 4ul of a 2ug/ul solution stabilised with ascorbate (0.2 mg/ml). HRP was injected into the caudate nucleus ipsilateral to the 6-OHDA lesion 7, 21, and 90 days post SN lesion as 0.05ul of a 30% solution. Spontaneous rotation was monitored with rotometers and the sensory-motor responsiveness of the animal was tested using olfactory, tactile or visual stimuli. Righting reflexes were also tested. Control studies with HRP injected into the caudate of an unlesioned animal revealed between 1 and 5 cells per brain which could be classified to have arisen from the contralateral ventral mesencephalon. Between 7 and 21 days after either the KA or 6-OHDA lesion of the SN, the numbers of cells within the contralateral ventral tegmentum had risen to between 1 and 17, typically 6 or 7. After 90 days the contralateral cell counts had returned to control levels. The circling induced by the 6-OHDA lesion varied between 20 and 100 turns per hour for the first four days, although stress induced rotation (tail clip) was apparent for at least 90 days. KA lesions of the SN resulted in mainly contraversive circling except for the first few hours after surgery. No motor asymmetries or sensory-motor lateralisation within the modalities tested were apparent upon testing 12 to 90 days post lesion. Amphetamine induced rotation was measured 21 and 90 days post lesion. Of a group of 16 rats, 6 of 7 KA lesioned animals, and 4 of 9 6-OHDA lesioned animals showed ipsilateral rotation 21 days post SN lesion. After 90 days 2 of 5 KA lesioned animals and 3 of 5 6-OHDA lesioned animals turned ipsilaterally, the others turning contralaterally or exhibiting a lack of responsivity to d-amphetamine at 2mg/Kg. The circling data of the above study is

of dubious value because KA would have lesioned the SNR glutamate sensitive neurones while sparing the SNC DAergic cells and their crossed projections. The KA lesioned subjects would therefore have represented an impaired striatal output, while the 6-OHDA lesioned subjects represented an impaired striatal input preparation, and as such are not strictly comparable. In the latter, the striatum would have been DA depleted and its function would have been impaired, while in the former, striatal function would have been normal. The locus of the lesion would be to impair the output of an intact striatum with a KA lesion of the SNR.

In a further series of experiments an increase in labelling of cells in the contralateral ventral mesencephalon with the retrograde fluorescent tracers Fast Blue and Nuclear Yellow was observed following 6-OHDA treatment of the ipsilateral SN as in the first experiment. There was no significant difference between the uptake of label by contralateral cells with either the fluorescent dyes or HRP (Pritzel et al. 1983).

Pritzel et al. (1983) suggest that reactive synaptogenesis of the crossed projection occurs in response to ipsilateral deafferentation, "...the denervation of the thalamus or caudate provides space for the proliferation of intact axon terminals from mirror image sites in the SN of the contralateral hemisphere". This process occurs concomitantly with recovery from lesion induced sensory-motor asymmetry and is complete within 1 week. The lack of contralateral labelled cells 90 days post lesion is difficult to explain. It suggests that the reactive synaptogenesis

of the crossed projection posited by Pritzel et al. (1983) is transient. The correspondence between the increased contralateral labelling and the cessation of spontaneous lesion induced circling suggested that the crossed projection may be functionally involved in the recovery from the lesion-induced motor asymmetry. The experiment of Pritzel, et al. (1983) thus suggested a testable functional role for the crossed projection in recovery from sensory-motor asymmetry as indicated by circling behaviour.

1.10 THE APPLICATION OF ROTATING RAT MODELS TO BASAL GANGLIA PATHOLOGIES.

Parkinson's disease is a slow progressive neurodegenerative disorder characterised by the loss of neurones from the DAergic cells of the SN (Langston 1985). Various attempts have been made to create animal models of this movement disorder (Pycock 1983) in order to research its nature and to evaluate anti-Parkinsonian treatments. Akinetic Parkinsonian-like states can be produced in rodents by striatal DA depletion pharmacologically or with lesions of the NS system. The rauwolfia alkaloid, reserpine, induces striatal DA depletion by its general monoamine depleting action when administered systemically (Carlsson et al. 1957, Goldstein et al. 1975), but this pharmacological model is clearly deficient due to its non-specific monoamine depleting action.

The use of 6-OHDA to specifically lesion the DAergic cells of the ventral mesencephalon, thereby uni- or bilaterally depleting the striatum of DA, produces the closest analogue of Parkinsonism in the rat. The resulting lesion is specific both for DA and the NS pathway. The bilaterally lesioned animal responds by exhibiting akinesia and catalepsy. This poses both an ethical and maintenance problem due to aphagia and adipsia as a result of this procedure. Unilateral lesions produce a good analogue for hemi-Parkinsonism. Hefti et al. (1980) have shown that partial unilaterally lesioned animals represent the best analogue of Parkinson's disease in the rat. Such an animal shows unilateral neglect syndromes, and

rotates ipsilaterally with amphetamine. There is a compensatory increase in the striatal DA/tyrosine hydroxylase ratio, which has also been reported in the human condition.

Recently, a group of young heroin addicts developed severe and irreversible Parkinsonian-like symptoms including bradykinesia, tremor and rigidity. Even detailed Parkinsonian symptoms like seborrhea and micrographia were present. Analysis of the drug sample used by these patients revealed a tentative culprit in MPTP. The conclusion was that MPTP was capable of producing, in humans, a condition which is almost totally indistinguishable from Parkinsonism. Treatment of these unfortunate individuals proceeded, with success, using standard anti-Parkinsonian remedies like L-DOPA. Non-human primates showed the same susceptibility as humans, although rodents appeared refractory to the neurotoxicity of MPTP. MPTP selectively affects the cells of the SNC, seemingly leaving the cells of the VTA intact. This implies differences in the membrane receptor characteristics of cells within the SNC and VTA. The lack of effect in rats suggests that rat ventral mesencephalic cells do not possess uptake mechanisms specific for MPTP.

Claims for MPTP inducing a more realistically Parkinsonian lesion in the primate than 6-OHDA in the rat, could be species specific rather than due to a further morphological specification of cell type. Animals with more complex CNS seem to be MPTP susceptible, but it may be the complexity of the nervous system rather than the lesion type that results in observation of Parkinsonian symptoms.

Histologic studies in one post-mortem human brain and other non-primates have shown there to be a swelling and distortion of the axons of the NS tract with a loss of terminals in the striatum and cell somata in the nigra. Tyrosine hydroxylase staining in the striatum was also found to be attenuated. However, these are rather global indices of NS lesion, and provide no clue as to the reason why MPTP most accurately mimics the disease in humans and other non-primates. Elucidating its mechanism of action, and how this differs from the action of other neurotoxins may shed light on the mechanism of cell death in this ideopathic condition. The cell death caused by MPTP is selective within the framework of the ventral mesencephalic DA cells. Only the cells of the SNC seem to accumulate the neurotoxin, and the vast cellular masses in the medial VTA are not affected (Langston 1983). This does not correlate well with the late stages of Parkinson's, in which mesolimbic DA depletion is also apparent. This brings to attention the progressive degenerative nature of the condition. The 6-OHDA lesioned syndrome does not demonstrate progressive degeneration. Thus Parkinson's disease seems to be a nerve membrane disorder, with the molecular nature of lesioning becoming an important research front. This, however, is outside the applicability of the circling rat paradigm.

The circling rat model is not intended to be an accurate model of the Parkinsonian condition at the biochemical level. In its basic 6-OHDA lesioned form the circling rat model is useful for screening potential anti-Parkinsonian DAergic drugs. The site of

action of the drug, whether pre- or postsynaptic, and dose-response relations to other well known DAergic drugs can be established in a cost-effective way. The experimental paradigm is also useful for research into other neurotransmitters involved in the Parkinsonian syndrome. The clinical finding of decreased glutamate decarboxylase, a GABA synthesising enzyme, and reduction of high affinity GABA-binding sites within the SN (Pycock 1983, Lloyd et al. 1977) in Parkinsonian brains, can be understood as a sequela of decreased DAergic function. GABA, as the major neurotransmitter of the output systems of the striatum, is involved in motor behaviour via VMT and midbrain sites. GABA levels decrease in response to diminished DAergic activity in the striatum. The change in binding affinities in the SN is unexpected according to the logical pattern of transmitter/receptor dynamics. Striatal DA depletion results in upregulation of DA receptors. The decrease in GABA affinities suggests a membrane effect, possibly decreased metabolic capability.

VMT lesions have been demonstrated to improve the rigidity component of Parkinson's. As determined with the rotating rat paradigm, an activity/catalepsy component is associated with striatal output to the VMT. Tremor in the Parkinsonian syndrome is likened to the aimless stereotypic behaviours evoked by DA agonists at the striatal level, GABA agonists at the nigral level, and GABA antagonists at the midbrain "angular complex" level. The component elements of the Parkinsonian syndrome are thus present in the rat. There are, however, several inconsistencies. The MPTP lesion, which in primates induces Parkinsonian symptomology,

affects the SNC cells and not the VTA cells. The 6-OHDA lesioned rat exhibits ipsilateral circling and posture when complete lesions of the ventral mesencephalic DA cells are made, or when the VTA/medial SNC is affected. Lesions specific to the SNC do not induce postural and locomotor asymmetry (Hodge and Butcher 1977). The greater simplicity of the rat CNS, while inducing a "low-pass filtering" of the Parkinsonian symptomology, is certainly no disadvantage when dealing with already global concepts like neurotransmitter levels and their relation to nerve nuclei and their interconnecting conduction pathways. As to the biochemistry and pathology of Parkinson's disease, to determine why the MPTP syndrome is not inducible in rats would reciprocally describe its action in primates, without the severe ethical and financial cost of primate research.

2.0 GENERAL METHODS

2.1 ANIMAL BEHAVIOUR AS AN INDEX OF THE PERFORMANCE OF NEUROCHEMICALLY AND ANATOMICALLY DISTINCT BRAIN AREAS.

2.2 MONITORING ROTATIONAL BEHAVIOUR.

2.2.1 THE ROTOMETERS.

2.2.2 DATA LOGGING.

2.2.3 COMPUTER PROGRAMS.

2.3 GENERAL SURGICAL METHODS.

2.3.1 HRP PROCEDURE.

2.3.2 6-OHDA LESIONING.

2.4 HISTOLOGY.

2.4.1 NEUROANATOMICAL TRACT TRACING WITH HRP.

2.4.2 HRP HISTOCHEMISTRY.

2.4.3 CRESYL VIOLET CHROMATIN STAINING PROCEDURE.

2.1 ANIMAL BEHAVIOUR AS AN INDEX OF THE PERFORMANCE OF NEUROCHEMICALLY AND ANATOMICALLY DISTINCT BRAIN AREAS.

Animal behavioural testing is usually associated with evaluation of psychological parameters, like conditioned appetitive or aversive behaviours. This sort of assessment is a "black box" approach. The study of neuropsychology attempts to relate animal models of psychological concepts, derived from human studies, to neurochemical and anatomical substrates. As a factual basis for this endeavour, it uses the observation of clearly characterised, reproducible behaviours, combined with experimental manipulations like lesioning of anatomically distinct nuclei and administration of drugs specific for neurochemically defined brain areas. The use of drug motivated behaviour, eg amphetamine induced hyperactivity and stereotypy, complicates the specification of behaviour to particular neural systems. Drugs may affect neurotransmitters in parallel or repercussively. Amphetamine releases both DA and NA, a parallel effect, while there are repercussive effects of changes in DA neurotransmission on acetylcholine (Lahue 1981). Drugs with subtle structural and biochemical differences, eg amphetamine and methylphenidate, induce differentiable behaviours. Methylphenidate induces gnawing stereotypy, while amphetamine induces mainly sniffing. This may be due to differences in the receptor subtype involved.

There are additional pharmacodynamic factors which should be taken into consideration. The route of drug administration affects

certain temporal parameters of drug action. Onset of drug effect and time until peak effect are grossly determined by route. The intraventricular route is the quickest, while subcutaneous and intramuscular routes are slower in onset and time till peak response. The temporal parameters of the intraperitoneal route are midway between the intraventricular and subcutaneous routes. The influence of the microanatomy of the CNS vascular system in the form of the blood brain barrier differentially affecting the rate of drug entry into the cerebrospinal fluid is another consideration when comparing the effects of different drugs. These effects, as well as peripheral receptor agonism, are bypassed by intra-ventricular or intra-cranial injection.

Circling behaviour differs from most psychometric behaviours as it is more clearly defined physiologically. The behaviour, spontaneously or pharmacologically driven, is an extrinsic manifestation well correlated with the intrinsic DA or GABA status. This simplicity precludes many of the difficulties associated with behavioural measures, although those associated with drugs remain.

Another system of behavioural observation is neurological testing of sensory-motor function before and after brain lesion. These tests usually involve observation of the animal's motor responses to various procedures designed to actuate specific perceptual modalities. Relevant to this study is the lateralisation of response to stimulation. The concept has to be used in its most general sense, however, as the relation of sensation of a stimulus

to the motor response indicating its detection is not a simple one. Unilateral lesion of the NS system gives rise to a behavioural syndrome considered to be contralateral sensory neglect (Feeney and Wier 1979). It is difficult, however, to assign the deficit unequivocally to an impairment of the sensory system, as non-response could be due to motor impairment, or an inability to sequence and co-ordinate a motor response.

A typical neurological examination is exemplified in Pritzel et al. (1983). Observation of orientation to olfactory, tactile, and visual stimulation was examined by presenting an acetic acid soaked cotton bud on either side of the head with and without visual cues and whisker tweaking. Righting reflexes were also tested. In this study, neurological tests were performed on unilaterally 6-OHDA lesioned animals 12 and 24 hours post-surgery, and before each measurement of rotational behaviour. Animals were removed from their cage sequentially, and placed in an open space. They were then rated as to whether impairment was present, its severity, and whether there was any improvement over the last test. The criteria investigated were; 1) Locomotion/exploratory behaviour

- 2) Orientation to sound ipsi- and contralateral to the lesion
- 3) Orientation to whisker tweaking ipsi- and contralateral to the lesion.
- 4) Orientation to ruffling of the fur ipsi- and contralateral to the lesion.
- 5) Orientation to a noxious olfactory stimulus in the form of an acetic acid soaked cotton bud presented ipsi- or contralateral to

the lesioned side. The term "orientation" was taken to mean an appropriate motor action elicited by the stimulus so as to investigate the stimulus, and to avoid it if it was noxious. In addition a brief description of the animals general orientation to its environs, presence or lack of contralateral limb neglect, ability to reverse direction when an immovable object was placed against the animal's side ipsilateral to the lesion, and condition of righting reflexes were noted.

2.2 MONITORING ROTATIONAL BEHAVIOUR.

Methods for measuring rotational behaviour vary. Observation of the animals on a flat surface and counting the number of complete 360 degree turns to the left or right is often encountered. Counting is usually done on a sample basis, typically 1 or 2 mins every 10 to 15 (Martin et al. 1978). Automated, continuous counting in specifically designed rotometers is a more sensitive, exact way to record circling. This has the advantage of decreasing the variance between trials. The design of a continuous recording environment falls into two classes. 1) Mechanical connection of the animal to the rotometer. 2) Optical designs such as have been developed by Hodge and Butcher (1978). In the latter, a replica of the animal's home cage was constructed with a central pillar emitting a radial pattern of 6 infra-red light beams in a horizontal plane approximately half the rat's height above the floor of the cage. Six detectors were located in the walls of the cage. By interrupting the beams with its passage around the pillar, the animal encoded a microprocessor which could determine

its rotation direction and rate. The advantage of this system is lack of restraint induced by the harness and coupling necessary in the other design. Habituation to the harness does, however, minimise the restraining drawback.

A commonly used mechanical rotometer design is that of Ungerstedt and Arbuthnott (1970). This system consists of a perspex hemisphere with a cam and micro-switch arrangement supported overhead at the geometrical centre of the sphere. The rat is placed in the bowl and connected to the cam by a length of steel wire and a harness. The effect of the hemisphere would be to accentuate rotation and diminish exploratory behaviour and stereotypy. This would tend to maximise the rotating rat paradigm as an assay of DAergic drugs.

2.2.1 THE ROTOMETERS.

The rotometers used in this study consisted of plastic cylinders of 40cm height and 30cm diameter. Across the top of this was mounted a wooden bridge which carried the sensing device. This in turn was connected to a harness for the attachment of the rat by means of a flexible wire. The rotometer was placed on a flat cardboard surface. The cylinder was opaque, thus reducing visual interference. The optical sensing device consisted of a perspex disc encoded by opaque transfers into quadrants (fig. 2.1). The photocells were mounted 180 degrees apart. The disc was damped to suppress noise generated by disc oscillations between adjacent quadrants induced by the springiness of the connecting wire. The

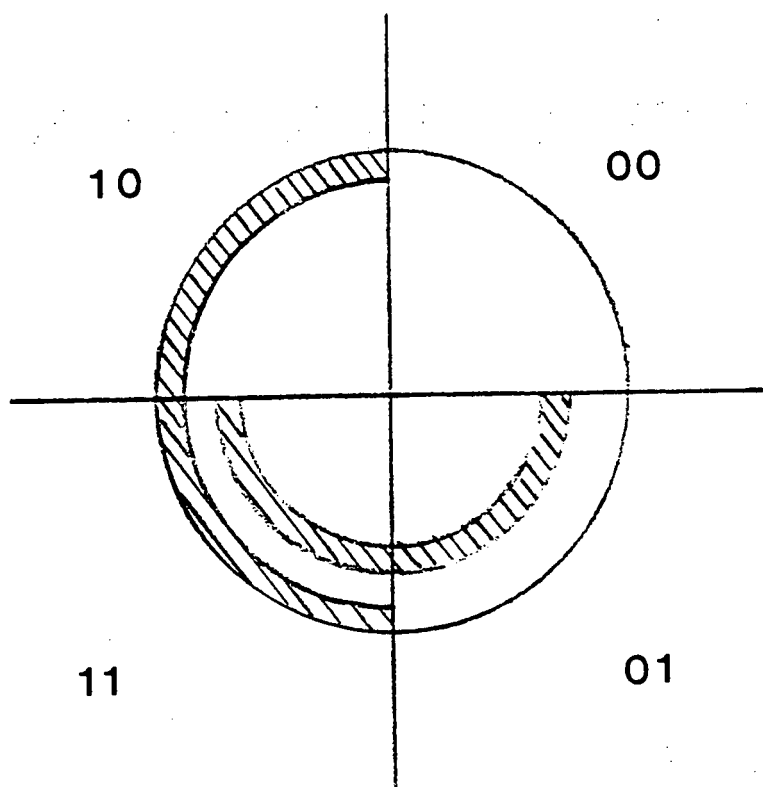
information generated by the optical sensing device consisted of a 2 by 4 array of 5 and 0V line levels. A schematic illustrating the machine link up and logic is presented in fig. 2.2. Circuit diagrams of the Schmidt trigger and optical sensor are presented in appendices. The rotometers could record quarter turns to either direction made by the rat. This enabled three parameters to be generated. (a) Total rotations: the number of quarter turns made to either side. This is a measure of the animal's general motor activity. (b) Nett rotations: the number of quarter turns made to the dominant side. This represents a composite of activity and motor asymmetry. (c) The asymmetry index, ($AI = \text{nett} / \text{total} * 100$) was subsequently calculated.

2.2.2 DATA LOGGING.

The TTL nature of the optical sensor's output allowed its interface with a digital computer. A BBC-B (Beeb) microcomputer was used for data logging and processing. To achieve rotometer-experiment interface, the userport of the BBC was connected to the rotometers. The userport is a series of bit memory locations within the 6522 Versatile Interface Adaptor (VIA), providing 2 ports, handshaking, interrupts, 2 timers and a serial register. The VIA is located at memory locations &FE60 to &FE6F. Port "B", the one used, are the 8 bits at &FE60 connected to corresponding plug pins. A data direction register (&FE62) controls whether the &FE60 bits are input or output by programming a 0 or 1 into the corresponding data direction register bit. A 0 programs the relevant userport bit "input", a 1 programs "output".

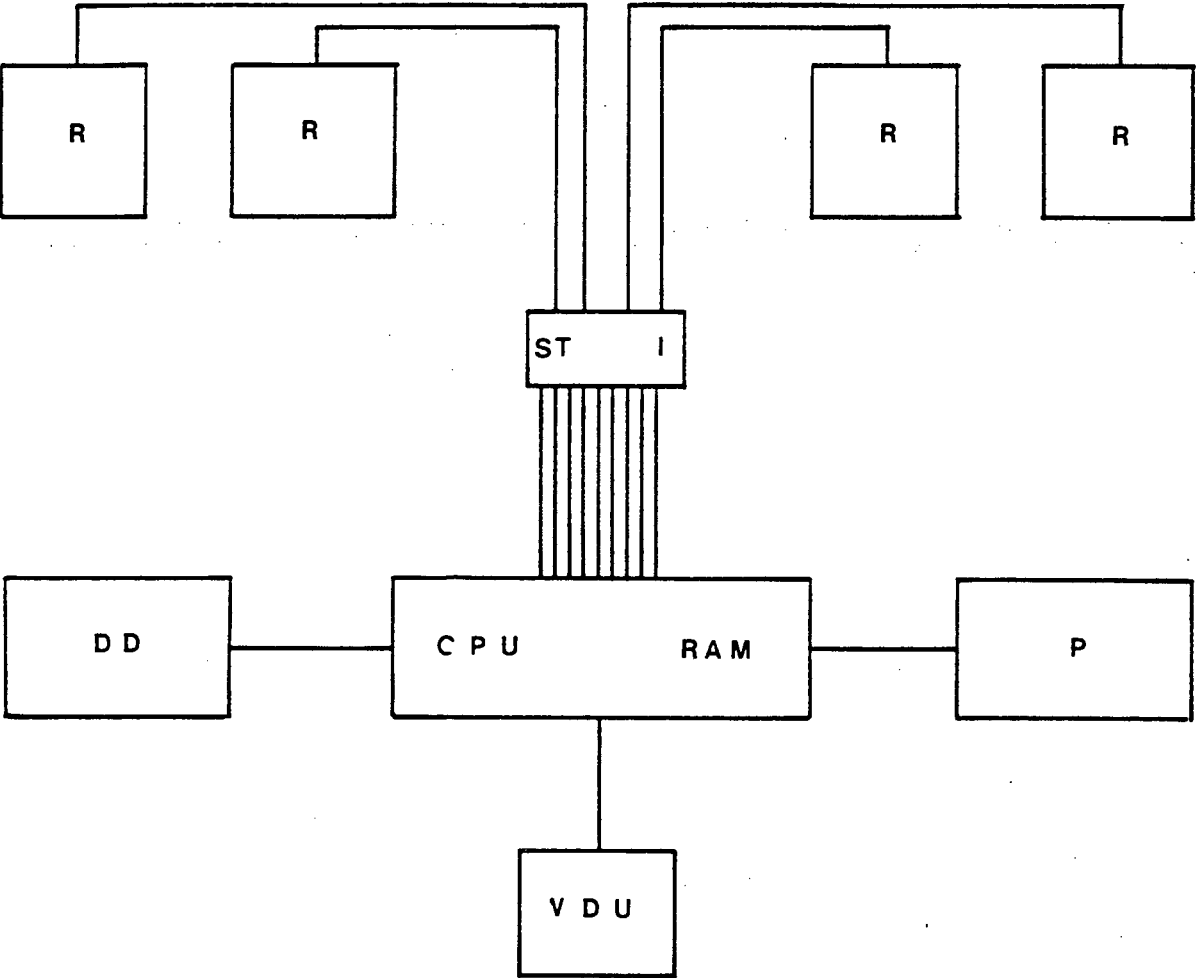
The data-logging program used interrupts triggering. The interrupt informs the program that a change has occurred in some peripheral device being monitored, in this case the rotometer. The program stores the machine state at a specific memory address, services the interrupt, and then restores the machine state. This system allows most efficient use of processing time. The cycle time of the data-logging program was about 200msec, sufficiently fast to allow 4 chambers to be monitored simultaneously (fig. 2.2).

FIG 2.1. Rotometer disc encoding. The disc is constructed from perspex, while the hatched region indicates an opaque transfer which divides the disc into quadrants. The binary code produced is indicated for each quadrant.



1 mm

FIG 2.2. Block diagram of the recording setup. R Rotometer, ST Schmidt trigger, I Interrupt generator, DD Disc drive, CPU Central Processing Unit, RAM Random Access Memory, P Printer, VDU Video Display Unit.



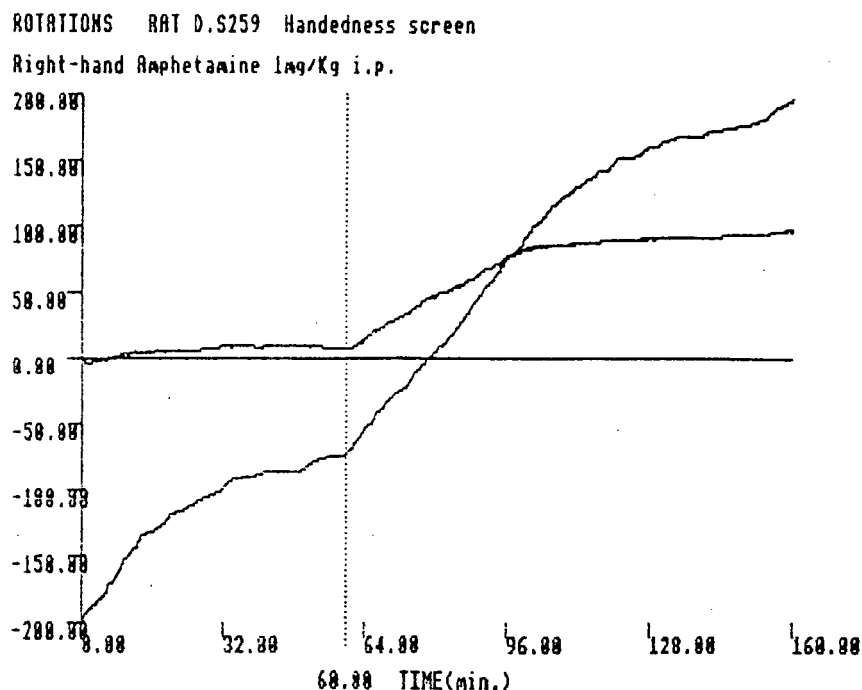
2.2.3 COMPUTER PROGRAMS.

Program "Timon"

This program monitors and records the status of rat rotometer chambers, and creates data files on disc. It uses an assembly language procedure (lines 350-590) to extract the relevant information from the userport byte. When the CB1 interrupt line undergoes a 0 to 1 transition, the procedure is called, and loads the contents of the userport to a specified memory address. The interrupt also stores the clock-value, which has been running from 0 since the start of the program, under "TIME". Successive two bit slices are removed from the userport byte, each representing the current status of one rotometer chamber. Each two bit slice is stored under a specific label. A status change of any of the rotometer chambers generates an interrupt. The current rotometer status is compared to that at the last interrupt, and by reference to a look-up table, the direction of rotation is determined. If a given rotometer's status has changed, the time since the last interrupt is stored in the array "RAT". The direction of rotation is encoded by a negative for left, positive for right, assigned to the clock-value of the interrupt. This allows for efficient disc-space utilisation, while retaining the data in a relatively raw, unprocessed form. The contents of the array "RAT" are written to disc files every 10 mins. A dual disc drive was used, and 4 files were opened simultaneously, one on each side of the discs.

Program "Integrt"

This program reads the files generated by "Timon". It integrates the number of clock values both with and without respect for sign. When integrated with respect for sign, nett rotations are generated. Nett rotations are a combined measure of the motor hyperactivity component of rotation, and the motor asymmetry component. Integration without respect for sign generates total rotations. Total rotations are a measure of the motor hyperactivity component of rotation. These integrals are plotted against time. Nett rotations are plotted from 0 midway up the Y-axis. Right rotations are above the X-axis, and left below. The origin of total rotations is displaced to the bottom of the Y-axis. An example of the graph drawn by "Integrt" for an unlesioned animal, #259, is presented below. The amphetamine (1mg/Kg, i.p.) injection is at 60 min. Nett rotations indicate that the animal rotated to the right. Total rotations are about 3 times greater than nett rotations, indicating that the animal was active, but not exclusively to the right; many left turns were also performed.



Program "Table"

This program tabulates the data recorded by "Timon". The routine tabulates to user-specified control period, response period, and binsize. It has default values of 60 min. control period, 70 min. response period, and 10 min. binsize. An example of the table produced is presented below. Accumulated turns are presented in the right block, while turns/10 min. bin are presented in the left block. Left, right, nett, total, and nett/total are tabulated in each block.

Harbress screen										
file: 0.0259 lesion: NA										
negative=left turns Amphetamine 1mg/Kg										
Turns/10min bin										
bin	left	right	nett	total	n/t	Accumulated turns				
						LEFT	RIGHT	NETT	TOTAL	N/T
Control:										
-25.00	1.75	2.00	0.25	3.75	0.07	1.75	2.00	0.25	3.75	0.07
-15.00	7.25	5.25	-2.00	12.50	-0.16	9.00	7.25	-1.75	16.25	-0.11
-5.00	10.25	31.00	20.75	41.25	0.50	19.25	38.25	19.00	57.50	0.33
70min						44.85	89.12	44.27	133.98	
Response:										
5.00	8.50	27.50	19.00	36.00	0.53	8.50	27.50	19.00	36.00	0.53
15.00	12.25	30.00	17.75	42.25	0.42	20.75	57.50	36.75	78.25	0.47
25.00	12.50	30.25	17.75	42.75	0.42	33.25	87.75	54.50	121.00	0.45
35.00	15.50	20.25	4.75	35.75	0.13	48.75	108.00	59.25	156.75	0.38
45.00	9.25	12.25	3.00	21.50	0.14	58.00	120.25	62.25	178.25	0.35
55.00	5.75	6.75	1.00	12.50	0.08	63.75	127.00	63.25	190.75	0.33
65.00	0.00	0.25	0.25	0.25	1.00	63.75	127.25	63.50	191.00	0.33

```

>L0L.
10 REM Program TIMON2B
20 REM This program monitors four rat rotometer chambers.
30 REM Each animal is connected to a spindle, on which is mounted an encoder disc.
40 REM The four sectors of the disc give rise to a 01320 binary sequence for clockwise rotation.
50 :
60 REM Rodney Douglas. Modified by G D Van Wageningen - UCT Med.School - July83
70 :
80 Q%=420206
90 DIM TABLE%(3,3),LAST%(3),CURRENT%(3),MVE%(3)
100 DIM PERIOD(3),RAT(1000,3),NPTS(3),FILE(3),ETIME(3)
110 :
120 DIM R$(3),DF(3),ERA(3),F$(3)
130 :
140 REM Look-up table for direction of movement. 9=error(2sector move)
150 :
160 TABLE%(0,0)=0:TABLE%(0,1)=1:TABLE%(0,2)=-1:TABLE%(0,3)=9
170 TABLE%(1,0)=-1:TABLE%(1,1)=0:TABLE%(1,2)=9:TABLE%(1,3)=1
180 TABLE%(2,0)=1:TABLE%(2,1)=9:TABLE%(2,2)=0:TABLE%(2,3)=-1
190 TABLE%(3,0)=9:TABLE%(3,1)=-1:TABLE%(3,2)=1:TABLE%(3,3)=0
200 :
210 REM ***** MAIN PROGRAM *****
220 :
230 PROCcompileROT
240 PROCInitialise
250 IF ?FLG=0 GOTO 250
260 ETIME=TIME
270 PROCUpdate
280 PROCstats
290 IF ETIME-TCHECK>60000 THEN TCHECK=ETIME:PROCdump
300 ?FLG=0:REM Reset interrupt service flag
310 GOTO 250
320 :
330 REM *****
340 :
350 DEF PROCcompileROT
360 REM This procedure compiles the interrupt routine .ROT
370 REM .ROT is called by a 0to1 transition on line CB1 of the user interface.
380 REM .ROT loads the current interface status, then strips off successive 2bit slices.
390 REM The four slices represent the current sectors of each of the four rotometers.
400 REM A sector change in any rotometer generates the hardware interrupt on CB1.
410 REM The sectors are returned in R0,R1,R2,R3.
420 REM .ROT sets FLG=1 to indicate to the BASIC program that an interrupt has been serviced.
430 :
440 ?&206=&00:??&207=&00:REM .ROT start address
450 R0=&70:R1=&71:R2=&72:R3=&73:STAT=&74:FLG=&75
460 FOR I%=0 TO 2 STEP 1:R%=&0000
470 COPTI%
480 .ROT
490 PHP:PHA:TXA:PHA:TYA:PHA
500 LDA &FE60
510 STA STAT
520 AND #3:STA R0
530 ROR STAT:ROR STAT:LDA STAT:AND #3:STA R1
540 ROR STAT:ROR STAT:LDA STAT:AND #3:STA R2
550 ROR STAT:ROR STAT:LDA STAT:AND #3:STA R3
560 LDA #1:STA FLG
570 PLA:TAY:PLA:TXA:PLA:PLP
580 RTI:1:NEXTI%
590 ENDPROC
600 :
610 DEF PROCInitialise
620 REM This procedure initialises the user interface, and zeroes some variables.
630 :

```

```

640 INPUT "Datafilename for Rat0 ",R$(0)
650 INPUT "Datafilename for Rat1 ",R$(1)
660 INPUT "Datafilename for Rat2 ",R$(2)
670 INPUT "Datafilename for Rat3 ",R$(3)
680 PRINT " Filenames OK? ";A$=GET$
690 IF A$(">") THEN GOTO 640
700 F$(0)=":"+"0"+"."+R$(0)
701 F$(1)=":"+"1"+"."+R$(1)
702 F$(2)=":"+"2"+"."+R$(2)
703 F$(3)=":"+"3"+"."+R$(3)
704 FOR X=0 TO 3
705 DF(X)=OPENOUT F$(X)
730 NEXT X
740 DUMMY=?&FE60:REM dummy read to reset user interface interrupt
750 ?&FE62=0:REM set all user interface lines to input.
760 ?&FE6E=&90:REM enable interrupts on user interface line CB1
770 ETIME=0:TIME=0:SLICE=0:TCHECK=TIME:FLG=0:ENDPROC
780 :
790 DEF PROCupdate
800 REM This procedure compares the current status of the rotometers with the
810 REM status at the last interrupt.
820 REM It uses a look-up table to determine the direction of rotation of each change.
830 REM If a rat has undergone a sector change, the time since the last change is written to the data array RAT.
840 :
850 CURRENT%(0)=?R0:CURRENT%(1)=?R1:CURRENT%(2)=?R2:CURRENT%(3)=?R3
860 FOR I=0TO3
870 MVE%(I)=TABLE%(LAST%(I),CURRENT%(I)):REM look up rotation direction
880 ETIME(I)=ETIME(I)+ETIME
890 IF MVE%(I)=0:GOTO 930
900 IF MVE%(I)=-1 THEN NPTS(I)=NPTS(I)+1:RAT(NPTS(I),I)=-ETIME(I):ETIME(I)=0:GOTO 930
910 IF MVE%(I)=1 THEN NPTS(I)=NPTS(I)+1:RAT(NPTS(I),I)=ETIME(I):ETIME(I)=0:GOTO 930
920 PRINT "*** ERROR *** Two sector move on channel ";I:PRINT
921 ERA(I)=ERA(I)+1
930 LAST%(I)=CURRENT%(I):ETIME(I)=0:NEXT:ENDPROC
940 :
950 DEF PROCstats
960 REM This procedure prints the current status of the rotating rats.
970 CLS
980 PRINTTAB(0,0)"ELAPSED TIME",ETIME/6000
1000 FOR I=0TO3
1030 PRINTTAB(I*8+10,12)RAT(NPTS(I),I)
1040 PRINTTAB(I*8+10,14)NPTS(I)
1050 NEXT:ENDPROC
1060 :
1070 DEF PROCdump
1080 ?&FE6E=&1C
1085 ON ERROR PROCchelp
1090 FOR R=0TO3
1120 FOR JJ=1 TO NPTS(R)
1130 PRINT#DF(R),RAT(JJ,R)
1135 PRINT JJ
1140 NEXT JJ
1141 NPTS(R)=0
1142 NEXT R
1145 ON ERROR OFF
1150 DUMMY=?&FE60:~&FE6E=&90:~FLG=0
1160 ENDPROC
1165 :
2000 DEF PROCchelp
2005 REM If an error arises in which
2006 REM the disc sector being written
2007 REM on is filled, this routine
2008 REM prevents the program from
2009 REM crashing by closing all files
2010 REM and opening new ones on empty
2011 REM sectors.

```

2015 ON ERROR OFF:CLOSE#0
2020 DF(0)=OPENOUT"Q1"
2030 DF(1)=OPENOUT"Q2"
2040 DF(2)=OPENOUT"Q3"
2050 DF(3)=OPENOUT"Q4"
2060 GOTO 1120
2650 INCREMENT=TIME/6000:TIME=0:ETIME=ETIME+INCREMENT:SLICE=SLICE+INCREMENT:PRINT ETIME

```

>LO.L
10 REM Routine for tabulating time-increment rotation data generated by the program TIMON2B or TIMON.
11 REM The routine can tabulate to user-specified control period, response
12 REM period and binsize without being phased (in or out).
13 REM User friendliness is good, without being ingratiating.
20 REM Rod Douglas and Gerhard Van Wageningen*
30 REM UCT Medical School 1984, 1985*
40 G%=&20209
50 DIM A(4000),left(15),right(15),accum(15),nett(15)
60 CP=0:RP=0:BS=0
70 :
80 REM***** MAIN PROGRAM *****
90 PRINT "input a comment line"
100 INPUT C$
110 INPUT "Enter control period in minutes; Press RETURN for 60 min. default";CP:IF CP=0 THEN CP=60
120 INPUT "Enter response time in minutes; Press RETURN for 70 minute default";RP:IF RP=0 THEN RP=70
130 INPUT "Enter binsize in minutes; Press return for 10-minute default";BS:IF BS=0 THEN BS=10
140 INPUT "Drug > ",D$
150 INPUT "Enter datafile name : ",F$
160 INPUT "side of lesion ",L$
170 INPUT "injection time > "Tinj
180 PROCclear
190 PROCload
200 PROCbin
210 CLS
220 VDU2:PROCprint:VDU3:GOTO 150
230 END
240 REM*****
250 :
260 DEF PROCload
270 Y=OPENUP F$:
280 Tstart=Tinj-CP
290 REPEAT:PROCread:UNTIL Trot>Tstart
300 A(1)=0:A(2)=Tcorrect:N=2
310 REPEAT:N=N+1
320 PROCread:A(N)=Tcorrect
330 UNTIL EOF#Y
340 NMAX=N-CLOSE#Y
350 ENDPROC
360 :
370 DEF PROCbin
380 N=0
390 REPEAT:N=N+1
400 Tbin=ABS(A(N))
410 BIN=INT(Tbin/BS)
420 IF BIN>15 THEN GOTO 470
430 accum(BIN)=accum(BIN)+.25
440 IF A(N)<0 THEN left(BIN)=left(BIN)+0.25 ELSE right(BIN)=right(BIN)+.25
450 IF A(N)<0 THEN nett(BIN)=nett(BIN)-0.25 ELSE nett(BIN)=nett(BIN)+.25
460 UNTIL N>NMAX
470 ENDPROC
480 :
490 DEF PROCprint
500 VDU1,27:VDU1,81
510 PRINT C$
520 PRINT "file: ";F$," lesion: ";L$
530 PRINT "negative=left turns ";D$;PRINT
540 PRINTTAB(14)"Turns/";BS,"min bin"TAB(68)"Accumulated turns"
550 PRINT " bin"," left"," right"," nett"," total"," n/t"," ", " LEFT"," RIGHT"," NETT"," TOTAL","
N/T"
560 PRINT"-----"
570 PRINT "Control:"
580 LEFT=0:RIGHT=0:NETT=0:ACCUM=0
590 FOR I=0 TO (CP/BS)-1

```



```

>LO.L.
10 REM Program INTEGRT
20 REM This program reads the data files generated by the rat-rotometer program TIMON2B.
30 REM Right (positive) and left (negative) quarter turns are added to yield the integrated number of full turns. This provides a measure of the animals asymmetry of movement.
40 REM The datafile is then re-read, and all quarter turns are added, irrespective of sign, to yield the accumulated number of full turns. This provides a measure of the animals activity without regard for asymmetry.
50 REM The integrated and the accumulated turns are plotted against time. The origin of the integrated turns is mid-way along the Y-axis. The origin of the Accumulated turns is displaced to the bottom of the Y-axis.
60 REM
70 REM Rodney Douglas - UCT Medical School - Jan 1984
80 :
90 REM ***** MAIN PROGRAM *****
100 :
110 MODE0:GCCL0,3
120 G% = 131594
130 PRINT "Enter a comment line for this data "
140 INPUT R$:PRINT
150 INPUT "Drug ",D$:
160 PROCassemble
170 PROCPARAMETERS
180 PROCAXES
190 PROCIntegrate
200 PROCaccumulate
210 PROCLabels
220 A$=GET$:
230 IF A$=" " THEN GOTO 270
240 IF A$="P" OR A$="p" THEN GOTO 260
250 GOTO 220
260 VDU2:CALL GDUMP:VDU3
270 CLS:CLG:GOTO 170
280 :
290 REM *****
300 :
310 DEF PROCPARAMETERS
320 PRINT "After the plotting on the screen the graph may be dumped to the printer by typing 'P'. Pressing the SPACE bar bypasses the dump."
330 PRINT:PRINT
340 INPUT "Enter filename "N$:
350 INPUT "Is this file pre AS30? (1=y) > ",FLG
360 INPUT "YMAX= ",YMAX
370 INPUT "INJ.AT? > ",U
380 INPUT "XMAX= ",XMAX
390 ENDPROC
400 :
410 DEF PROCAXES
420 CLS
430 VDU29,100,100;
440 XSCALE=1000/XMAX
450 YSCALE=800/YMAX
460 VDU5
470 PLOT 4,0,800:PLOT 5,0,0
480 REM X AXIS TICS
490 FOR X=0 TO 1000 STEP 200
500 PLOT 4,X,0:PLOT 5,X,-20:PLOT 4,X,-50:VDU11:PRINT;X/XSCALE;
510 NEXT
520 REM Y AXIS TICS
530 VDU 29,100,500;
540 MOVE 0,0:DRAW 1000,0
550 FOR Y=-400 TO 400 STEP 100
560 PLOT 4,0,Y:PLOT 5,-20,Y:PLOT 4,-100,Y:PRINT;Y/YSCALE;
570 NEXT
580 ENDPROC
590 :
-----

```

```

600 DEF PROCIntegrate
610 Y=0:T=0
620 D=OPENUP N$
630 MOVE 0,0
640 REPEAT
650 INPUT#D,A
660 IF FLG=1 THEN T=T+ABS(A): ELSE T=ABS(A)/6000
670 DRAW T*XSCALE,Y*YSCALE
680 IF A<0 Y=Y-0.25 ELSE Y=Y+0.25
690 DRAW T*XSCALE,Y*YSCALE
700 UNTIL EOF#D OR T>XMAX
710 CLOSE#D
720 ENDPROC
730 :
740 DEF PROCaccumulate
750 Y=0:T=0
760 D=OPENUP N$
770 VDU29,100,100;
780 MOVE 0,0
790 REPEAT
800 INPUT#D,A
810 IF FLG=1 T=T+ABS(A) ELSE T=ABS(A)/6000
820 DRAW T*XSCALE,Y*YSCALE
830 Y=Y+.25
840 DRAW T*XSCALE,Y*YSCALE
850 UNTIL EOF#D OR T>XMAX
860 CLOSE#D
870 VDU29,100,500;
880 ENDPROC
890 :
900 DEF PROCLabels
910 VDU4
920 PRINTTAB(0,0) "ROTATIONS  RAT ";N$;"  ";R$
930 PRINTTAB(0,2) "Right-hand ";D$;" i.e."
940
950 VDU5
960 MOVE 450,-470
970 PRINT"TIME(min.)"
980 MOVE U*XSCALE,400
990 PLOT29,U*XSCALE,-430
1000 MOVE U*XSCALE-40,-470:PRINT;U
1010 ENDPROC
1020 :
1030 DEF PROCassemble
1040 REM DUMPS MODE0 OR MODE4 SCREEN ONTO CATCH 8510A PRINTER
1050 REM EXTENSIVELY MODIFIED FROM BIENBAUM 1982.
1060 REM ROD DOUGLAS - UCT MED.SCHOOL - 1983
1070 REM
1080 COLUMNS=&70:ROWS=&71:COLCOPY=&72:LIMIT=&73:BEGINCONTROL=&74:LOCATION=&75:STORE=&77:OSWRCH=&7FE
E:OSBYTE=&7FF4
1090 SMODE=&355
1100 FOR IX=0 TO 2 STEP 2:PX=TOP+1000
1110 IOPTIX
1120 .GDUMP
1130 LDA #&34
1140 JSR OSBYTE
1150 STX LOCATION
1160 STY LOCATION+1
1170 LDA SMODE
1180 CMP #4
1190 BNE ZEROMODE
1200 .FOURMODE
1210 LDA #20
1220 STA LIMIT
1230 LDX #7
1240 JSR CONTROL
1250 LDA #2A

```

```

1260 STA LIMIT
1270 LDA #20
1280 STA BEGINCONTROL
1290 LDA #40
1300 STA COLUMNS
1310 LDA #32
1320 STA ROWS
1330 BNE BEGIN
1340 .ZEROMODE
1350 LDA #13
1360 STA LIMIT
1370 LDX #0
1380 JSR CONTROL
1390 LDA #32
1400 STA LIMIT
1410 LDA #26
1420 STA BEGINCONTROL
1430 LDA #30
1440 STA COLUMNS
1450 LDA #32
1460 STA ROWS
1470 .BEGIN
1480 LDA COLUMNS
1490 STA COLCOPY
1500 LDX BEGINCONTROL
1510 JSR CONTROL
1520 .LOOP1
1530 LDY #7
1540 .LOOP2
1550 LDA (LOCATION),Y
1560 STA STORE,Y
1570 DEY
1580 BPL LOOP2
1590 LDY #8
1600 .LOOP3
1610 LDX #7
1620 LDA #1
1630 JSR OSWRCH
1640 .LOOP4
1650 ASL STORE,X
1660 ROL A
1670 DEX
1680 BPL LOOP4
1690 JSR OSWRCH
1700 DEY
1710 BNE LOOP3
1720 LDA LOCATION
1730 CLC
1740 ADC #6
1750 STA LOCATION
1760 BCC NOCARRY
1770 INC LOCATION+1
1780 .NOCARRY
1790 DEC COLCOPY
1800 BNE LOOP1
1810 LDA #1
1820 JSR OSWRCH
1830 LDA #80D
1840 JSR OSWRCH
1850 DEC ROWS
1860 BNE BEGIN
1870 LDA #42
1880 STA LIMIT
1890 LDX #32
1900 JSR CONTROL
1910 RTS

```

```

1920 .CONTROL
1930 LDA #1
1940 JSR OSWRCH
1950 LDA TABLE,X
1960 JSR OSWRCH
1970 INX
1980 CPX LIMIT
1990 BNE CONTROL
2000 RTS
2010 .TABLE:INEXTI%
2020 FOR I%=1 TO 42
2030 READ ?P%
2040 P%=P%+1:NEXT I%
2050 DATA 27,81,27,76,48,50,48,27,34,49,54,27,62,27,78,27,76,48,49,48,27,83,48,51,50,48,27,83,48,54,52,48,27,55,27,78,27,7
6,48,48,48,13
2060 ENDPROC

```

2.3 GENERAL SURGICAL METHODS

Rats were placed in a chamber pre-filled with ether fumes obtained by bubbling 95% O₂, 5% CO₂ (carbogen) through anaesthetic ether (Natal Cane Byproducts) via a Boyle's apparatus. Surgical anaesthesia was induced within 5 minutes, after which the animals were removed and placed in a stereotaxic instrument (Kopf) aligned to the de Groot system of co-ordinates, ie. incisor bar 5mm above the inter-aural line. Anaesthesia was maintained by supplying the subject with ether-carbogen via a Boyle's apparatus and face mask. The head was shaved and disinfected with 70% alcohol prior to surgery. The cranium was exposed by a midline incision 5mm behind the eyes and extending caudally for 20mm. The tissue overlying the cranial bones proper was retracted and bleeding was stopped by pressure application of a swab. In cases of severe bleeding, bone wax (Ethicon) was forced into the bleeding sinuses. A burr hole was drilled in the skull overlying the caudate or SN with an electric drill (Dremmel Motor Tools) fitted with a 0.5mm diameter burr at appropriate co-ordinates derived from a stereotaxic atlas (Pelligrino et al. 1979). The finished burr hole was approximately 1mm in diameter and extended through the cranium so that a paper thin thickness of bone was left and the dura was intact. A 26G needle was used to penetrate this bone cover and the dura precisely at the point at which the infusion cannula was to penetrate. The infusion cannula was lowered onto the dura with the cannula tip just touching the dura and the vertical co-ordinate was noted. The depth co-ordinate was calculated from this value.

2.3.1 HRP PROCEDURE.

2ul HRP (4% Sigma) was infused into the caudate at AP=7.8 LAT=2.8 and DEPTH=4.8mm calculated from the dura co-ordinate measured earlier. Infusion was from a 10ul Hamilton syringe connected to the cannula via a short length of polythene tubing. The 2ul volume of HRP was infused over 5 minutes and the cannula was left in situ for a further 5 minutes. This precaution, as well as gentle insertion and retraction of the cannula, served to minimise damage to the striatal cells and ensure maximal spread of the tracer throughout the entire nucleus. The surgical wounds were carefully examined for bleeding before closure with silk suture. Identical surgical procedures were used for HRP and 6-OHDA infusion.

2.3.2 6-OHDA LESIONING

Intracerebral injection was introduced by Ungerstedt (1971) in order to obtain focal depositions of 6-OHDA located within specific catecholamine pathways. This procedure has become standard practice, and involves the injection of 1-10ug 6-OHDA dissolved in saline or water, with 0.2mg/ml ascorbate to prevent autoxidation. 0.25-4ul volumes are injected at a rate of 1ul every 1-5min.

The size of the lesion produced depends on the concentration of 6-OHDA used, volume of vehicle, and the rate of infusion. The concentration of 6-OHDA affects mainly the intensity and hence

specificity of neurotoxicity, while the volume and rate of infusion affect the extent of diffusion from the deposition site. Faster infusion rates result in more extensive diffusion as well as non-specific tissue damage due to pressure distortion. There is more controversy over the extent of neurotoxic specificity with intracerebral routes of infusion than with the others. Histological examination of a representative lesion site reveals 3 or 4 distinctive, definable zones. These are generally agreed upon and were confirmed in this study. 1) The cellular disruption produced by the passage of the infusion cannula. 2) The astrocytic walling off of the cannula track. 3) The focus of the lesion site where non-specific cellular destruction has occurred (determined mainly by the concentration 6-OHDA used). 4) A region of specific cellular damage extending spheroido-radially out from the injection focus, shading imperceptably into healthy tissue. This zone is determined by the extent of diffusion.

6-OHDA specificity is dependent on the existence of a specific uptake mechanism. NA fibres are more sensitive to 6-OHDA than are DA fibres. This complicates the issue of obtaining selective DA or NA lesions, although multiple low doses of 6-OHDA (intra ventricular) destroy NA cells while allowing the more robust DA cells to survive. DA neurone selectivity can be enhanced by desipramine pretreatment due to its NA depleting action (Breese 1975).

In this study, 6-OHDA was infused into the ventral mesencephalon

at various co-ordinates. Lateral SNC AP=3.0 LAT=2.8 D=7.2; ventral SN AP=2.4 LAT=1.0 D=9.6; DTV A=2.4 LAT=0.0 D=8.2; and anteromedial SNC AP=3.0 LAT=1.0 D=7.4 (Pelligrino et al. 1979). Infusion of 2ul 6-OHDA with ascorbate 0.2mg/ml occurred over 5 minutes, with a further 5 minutes allowed before cannula retraction.

2.4 HISTOLOGY.

2.4.1 NEUROANATOMICAL TRACT TRACING WITH HRP.

HRP is the peroxidase enzyme isolated in crystalline form from the root of the horseradish plant. Peroxidases are so named due to their exhibiting oxidising properties only in the presence of peroxides such as hydrogen peroxide. The HRP enzyme has a molecular weight of around 40000, and its diameter is 4.74 nm with a 0.3 nm hydration shell. It contains a heme group that is essential for its histochemical activity. When accompanied by its substrate, hydrogen peroxide, HRP is capable of catalysing the oxidation of chromogenic compounds and thus forming a visible reaction product.

The HRP molecule is sufficiently small that it diffuses through the extracellular space at a minimum rate of 0.5 mm/hr. It is endocytosed by cells and has been used in a system for monitoring the pinocytosis of macromolecules into kidney cells (Straus 1957 cited by Mesulam 1982) and neurones (Becker et al. cited by Mesulam 1982) from the extracellular space. The use of HRP as a retrograde tracer of neuronal interconnections was heralded in 1971 by Kristensson et al. (1971), and has since proven to be a reliable and sensitive method for positive identification of cell bodies by retrograde transport of tracer from their termination sites.

HRP may be released into CNS structures in a number of ways consistent with the goal and scale of the operation.

Ionophoretic release of HRP from micropipettes is employed when working at the single-cell level, and makes use of uptake mechanisms of the soma, or HRP can also be injected directly into the soma following intracellular puncture by the recording micropipette. HRP thus included in a cell can enable the visualisation of the morphology and arborisation of a cell from which electrophysiological data has been obtained. Though this technique is powerful and valuable, it will not be discussed further as it is not relevant to the current study.

Pressure injection of HRP is usually employed for the purposes of neuronal tract tracing. Chronologically, this technique was developed first, and a number of procedural variations have been developed to date. There are several factors which can influence the apparent extent of the HRP deposition site, and practical considerations have necessitated the definition of an effective, and a virtual injection site. This has great relevance in retrograde tract tracing as localisation of the uptake zone is vital for the interpretation of results. The concentration and volume of HRP injected are the two most important variables determining the extent of the deposition site, though the tissue characteristics, survival time of the animal, fixation, and the sensitivity of the histochemical technique employed to demonstrate the presence of peroxidase activity also exert an influence.

HRP is usually prepared for pressure injection in aqueous solution, often buffered, with, for example, tris KCl (pH 8.6). Mesulam describes the events at the site of tracer injection as belonging to three distinct phases. From 0 to 2 hr there is an initial distribution of tracer through the region of injection which is driven by the pressure of the volume introduced into the structure. The initial extent of the injection zone will depend on the volume injected and rate of infusion, with greater volumes and rates spreading the tracer further away from the region immediately surrounding the cannula orifice. A primary diffusion of tracer into the surrounding tissue will continue the expansion of the injection zone until about 10 minutes, and the zone remains constant in size until about two hours. The concentration of tracer injected will exert its greatest effect during the primary diffusion stage, with higher concentrations producing more extensive diffusion. Vanegas (1978 cited by Mesulam 1982) claims that HRP uptake into intact neuroglia occurs only within this two hour period, during which the extracellular space at the infusion site is bathed with HRP. HRP also enters neuronal elements ruptured by the cannula insertion and injection process, where it is sequestered into vesicles for retrograde transport. The second phase is termed reactive expansion, and there is an apparent expansion of the infusion zone as determined by the distribution of reaction product from 2 to 8 hours, followed by shrinking by 17-18 hours. Uptake for subsequent transport does not appear to occur during the period of reactive expansion. The reasons for the reactive expansion phenomenon are not understood, though immune responses in the form of increased vascular permeability and

macrophage activity may cause scattering of HRP debris. Furthermore, the initial transport through neural elements in the immediate vicinity of the infusion zone may play a role. During the third phase from 18 hours until death, there is a resolution of the reaction product deposition around the injection site, as shown by a gradual contraction after 18 hours due to the uptake of residual HRP into neuroglia and its subsequent degradation by enzymatic action.

The labelling of cell somata via retrograde transport along an afferent pathway is dependent on the endocytotic uptake of HRP and its subsequent transport in vesicular structures to the perikaryon. There are two descriptions of endocytosis, bulk endocytosis and adsorptive endocytosis.

Bulk endocytosis is an incorporation of vacuoles containing the unbound macromolecule into the cell by invagination of the cell membrane and subsequent fusion of the points of origin of the invagination. There is no active substrate concentration during the endocytotic process. The rate of bulk endocytosis is affected by the size and electrostatic charge of the macromolecule. Polycationic macromolecules of increasing molecular weights stimulate the incorporation of any macromolecules in the extracellular space. Another factor which increases the rate of bulk endocytosis is the degree of activity of the neurone.

Adsorptive endocytosis occurs with different dynamics to bulk endocytosis. Certain macromolecules (eg. wheat germ agglutinin)

bind to surface membrane receptors and are incorporated into the cell by pinching off of cell membrane into vacuoles with the receptor/macromolecule complex forming the inner surface. Adsorptive endocytosis is far more effective compared to bulk endocytosis, and occurs at far lower extracellular concentrations. There does not seem to be the same dynamic relationship between neuronal uptake and the degree of neuronal activity that is apparent with bulk endocytosis. The improved efficiency of HRP conjugated to wheat germ agglutinin ^(WGA-HRP) is attributable in part to its uptake by adsorptive endocytosis. The adsorption of the WGA-HRP to the membranes in the vicinity of the injection site would limit spread of tracer, minimising uncertainty in the determination of the effective injection site. Additionally, the improved sensitivity for both uptake and intraganglionic transport motivates the use of WGA-HRP over standard HRP. However, the performance of HRP is itself quite adequate for retrograde anatomical tracing, especially when combined with a suitably sensitive chromogen. Moreover, the cost of WGA-HRP makes its use an extravagance except for certain applications. These include anterograde tracing, high resolution intracellular punctures with micropipettes for illumination of complex, fine dendritic fields, or in confined uptake target zones where critically precise determination of the uptake zone is required.

Endocytosis occurs most frequently at axonal terminals and least frequently along the myelinated part of the axon, with uptake from dendritic terminals and the perikaryon membrane (Lavail and La Vail 1974). This is a critical observation as the specification of

the site of origin of HRP uptake is partly dependent on there being no uptake by undamaged fibre tracts passing through the injection site. Transport mechanisms operating within the dendrites are identical to those described for axonal transport, both in the retrograde and anterograde directions (73 mm per day anterograde; Kreutzberg et al. 1973, Schubert et al. 1972). Once tracer has been incorporated into the terminal it is transported to the perikaryon by a long loop mechanism responsible for the recycling of membrane components. The endocytotic organelles which are incorporated into the axonal terminals are modified during their passage down the length of the axon, fusing with other membranous structures, finally being transformed into perikaryal dense bodies incorporating lysosomal enzymes. In the perikaryon the membrane is broken down into its components and recycled, while the HRP degradation is rather more indolent; HRP remaining detectable in the soma until 5 days after the extracellular stores of tracer have been exhausted.

The specification of the site from which neuronal elements have taken up tracer is one of the most crucial aspects of HRP neurohistochemistry, and is dependent on certain assumptions which unfortunately have not been fully described. As tracer uptake mainly occurs from 15 minutes to two hours after injection (Mesulam 1982), the spread of tracer produced in the first phase would be the most accurate index of the region of effective uptake. Two hour survival times do not allow sufficient time for retrograde transport to have occurred, and a compromise in the form of a virtual infusion site must be adopted. As the virtual

injection site can vary considerably, careful notice should be taken of the parameters which influence its extent. Effective and virtual deposition sites are influenced equally by the pressure, volume and concentration of tracer injected. There are other parameters which affect the effective and virtual deposition sites independently, eg survival time, fixation, and histochemical development method.

Survival times of 2 to 17 hours may result in overestimation of the effective injection zone, while long survival times of 72 hours or more may result in its underestimation. The criteria for selecting survival time are that sufficient time be allowed for transport to the target site to have occurred, but which does not allow excessive degradation, such that the virtual and effective injection sites overlap.

Excessive exposure to aldehydes during fixation decreases HRP activity at the sites of injection as well as transport. Excessive fixation and lack of uniformity in the fixation protocol should be avoided.

Underfixation is the converse danger and can lead to instability of the reaction product, resulting in early degradation of the quality of the histology.

Histochemical procedures for demonstrating HRP vary greatly in sensitivity. The virtual injection site will thus vary with methods of varying sensitivity. The difficulties in assigning a

precise injection site with HRP neurohistochemistry have thus been clearly stated, and remain a major difficulty with the method. In practice, inter-experimental fluctuations can be limited by using suitable and identical injection procedures, survival times, and fixation schedules.

A typical virtual HRP injection zone visualised by a sensitive histochemical technique consists of a dense central core surrounded by a fainter halo. There is evidence for uptake from both core and halo, though some claim that the halo region is not one of active uptake. Although the latter may appear to be densely stained, it is argued that this is due to the outcome of reactive transport and reactive distribution. It is generally assumed that the densely stained central core is the injection site with the most significant amount of extracellular HRP confined to its borders. Thus, when a histochemical method of high sensitivity is used, the centre of the injection site is taken to be the region of effective uptake.

2.4.2 HRP HISTOCHEMISTRY

HISTOLOGY AND HRP NEUROHISTOCHEMISTRY

A survival time of 48 hours was allowed for uptake and transport of the tracer from the striatum to its mesencephalic destination. While under deep chloral hydrate anaesthesia the subject was exsanguinated with saline by rapid perfusion of a saline solution through an intra-cardial 15G needle. The needle was inserted into

the left ventricle and clamped in place with an artery forceps. Exsanguinate was released via an incision in the right atrium. Pressure head was supplied by a gravity feed of 2 metres. After 60 to 80 seconds of saline perfusion, the exsanguinate was a light orange colour and the animals' extremities were pallid. The saline perfusate was then replaced by a Karnofsky's solution, switched into circuit via a 3-way tap. Fasciculation followed by rigidity was taken as the sign of a good perfusion. Approximately 500ml of Karnofsky's was perfused over 20 minutes. Of the 500ml, 250ml were perfused rapidly, within 5 minutes.

The perfused cadaver was skinned above the shoulders and the head removed with a sharp scissors at the level of the second cervical vertebra. The cranium was removed with Rongeurs and the brain-skull was post-fixed for 24 hours in Karnofsky's. The brain was blocked by remounting the head in the stereotaxic apparatus and performing a coronal cut through the anterior cerebellum and brainstem. The brain was placed in 10% sucrose buffer until it sank.

Histological sections of 50 to 100µm thickness were cut on a freezing microtome (American Optical) and placed in ice trays filled with cold 0.1M phosphate buffer. Sections between AP=9.0 and AP=2.0 were saved for analysis.

Once the serial sections had been mounted and coverslipped, they were observed under the low power optic of an Olympus OM30 microscope. A grid (fig. 3.1A) was constructed, superimposed over

the section under observation by drawing an imaginary line through the centre of the section in the dorso-ventral plane. Further lines were constructed at intervals lateral to this centre-zero by means of an eyepiece mounted graticule. The line 1mm lateral to the zero reference line coincided with the point at which the SNC cell cap meets the ventral floor of the mesencephalon. The area subtended by the 1mm line and the zero line contained the cell groups VTA and DTV, and the 0.5mm line further subdivided the area into the DTV medially and the VTA laterally. The area between the 1 and 2.8mm lines contained the pars compacta of the SN. This cell counting rationale was employed in order to standardise cell counts for cell groups and between individual animals.

Where every section was saved and cell counts performed, sections were binned to the co-ordinates specified in the atlas of Pelligrino et al. (1979), eg cell counts of 50um sections between AP=3.0 and AP=2.8 were summed to the section closest to the Pelligrino section. The section closest to the Pelligrino section was identified in terms of correspondence between the size and location of major structures like the medial lemniscus, red nucleus (RN), SNC cell cap, and DTV, as indicated in the atlas compared to the experimental histology. Tables of cell counts were constructed as summed cell counts ipsi- and contralateral to the midline according to the grid pattern described earlier.

The Hanker et al. (1977) technique for developing the chromogenic HRP product was used as it provides adequate sensitivity while minimising the toxicity hazards associated with

tetra-methyl-benzidine. The sections were reacted in perspex chamber arrays which were loaded in sequential fashion from the ice trays. Reacted sections were mounted on gelatinised slides and air dried for 24 hours. Counter-staining with cresyl fast violet was possible at this stage, but was not carried out on a routine basis as it was easier to visualise the HRP filled somata without counterstain. For the purposes of performing cell counts the sections were dehydrated through alcohol to zylol, and coverslipped with DPX mountant (BDH). Slides so treated could at a later stage be counterstained with cresyl violet by removing the coverslips and DPX in zylol, and rehydrating the sections.

VISUALISATION OF HRP BY THE METHOD OF HANKER ET AL.

The following were weighed out into beakers.

A	nickel ammonium sulphate	0.8g
	cobalt chloride	1.2g
B	catechol	0.4g
	p-phenylenediamine diHCl	0.2g

Dissolve A in 200ml d-H₂O, B in 400ml cold cacodylate buffer, pH 5.1. Mix 2 parts A with 1 part B (initial H-Y). Transfer brain sections from 0.1M phosphate buffer to initial H-Y. Agitate for 15 mins. Wash for 2 mins in phosphate buffer. Add 1 drop 25% H₂O₂ to 300 ml cold B still remaining. Transfer sections from phosphate buffer to H₂O₂-B and agitate for 25 mins. The formation of reaction product should be monitored by visual inspection. Should

the reaction proceed for too long, the background stains darkly. The reaction should be terminated before it reaches this stage.

Phosphate buffer 0.2M pH 7.6

(a) $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (M.Wt 358.14) 71.62g/l 256.4g/3580ml.

(b) $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (M.Wt 156.01) 31.2g/l 35.9g/1150ml.

Mix 1150 ml (b) with 3580 ml (a) (or different volumes in the same ratio). Adjust pH to 7.6 with conc NaOH. Keep 0.2M stock in fridge and make up 0.1M as required.

Cacodylate buffer 0.1M pH 5.1

Sodium Cacodylate 21.5g.

Concentrated Hydrochloric acid +- 8ml.

Place beaker with cacodylate on stirrer and add approximately 900 ml dH_2O , then add acid. Adjust pH to 5.1 with conc. HCl (critical). Make up to 1 litre.

Acetate buffer 0.2M pH3.3

1M sodium acetate

1N HCl

Karnofsky's Fixative

Heat 1800ml dH₂O to 60C to depolymerise paraformaldehyde. Add 40g paraformaldehyde at 40 degrees C as H₂O cools. Add a few pellets NaOH until solution clears. Add 200ml 0.2M phosphate buffer. Adjust pH to 7.4-7.6. Immediately prior to use, add required amount of 50% Gluteraldehyde solution which should be refrigerated. Final concentration of gluteraldehyde must be 2.5%. Paraformaldehyde concentration is 1%. Karnofsky's is conveniently prepared 1l at a time, thus 950 ml of stock with 50ml of gluteraldehyde. Gluteraldehyde need only be practical grade.

10% buffered formalin

Formalin 200ml

Tap H₂O 1800ml

Na₂HPO₄ 13g

NaH₂PO₄*2H₂O 8g

2.4.3 CRESYL VIOLET CHROMATIN STAINING PROCEDURE.

1) Demyelination

dH ₂ O	30 sec
70% ethanol	"
96% ethanol	"
96% ethanol	"

absolute ethanol	"
absolute ethanol	"
zylol	10 mins
absolute ethanol	30 sec
absolute ethanol	"
96% ethanol	"
96% ethanol	"
70% ethanol	"
d-H2O	"

2) Cresyl violet staining

acid dH2O	30 sec
acid ethanol	"
70% ethanol	"
96% ethanol	"
96% ethanol	"
absolute ethanol	"
absolute ethanol	"
zylol	10 mins
coverslip with DPX mountant	

Cresyl fast violet

0.1% in Walpole's acetate buffer pH3.9.

CFV 0.2g

6g/l acetic acid 165ml

13g/l sodium acetate 35ml

3.0 HRP INVESTIGATION OF THE CROSSED MESOSTRIATAL SYSTEM.

3.1 INTRODUCTION.

3.2 CONFIRMING THE CROSSED PROJECTION, ESTABLISHING ITS EXTENT AND SITE OF DECUSSATION.

3.2.1 CONTROLS.

3.2.2 NORMALISATION RATIONALE, ESTABLISHING A STANDARD HRP DENSITY, CELL COUNT CURVE.

3.2.3 EVIDENCE FOR THE SITE OF DECUSSATION.

3.3 DISCUSSION

3.1 INTRODUCTION.

The NS system was described in section 1. A crossed mesostriatal projection has been described in the cat (Royce 1978) using HRP, and in the rat (Fass and Butcher 1981) using the retrogradely transported dye, Evans blue (EB). In the latter case, ipsilateral deposition of EB in the rostral caudate nucleus resulted in the ventral mesencephalic DA cells being labelled via the NS projection. Altar et al. (1983) and Fallon et al. (1983) conducted similar experiments in order to further characterise the crossed projection. However, uncertainty remained as to what proportion of the mesostriatal cells decussate, where they decussate, whether they contain DA, and the functional significance, if any, of the crossed mesostriatal projection.

In the current study the retrograde tracer enzyme HRP was used to investigate the crossed projection. The aims of the study were four-fold; 1) to confirm the existence of the crossed projection 2) to determine its site of decussation 3) to establish the extent of the crossed projection in naive animals so as to control experimental subjects with manipulated NS systems 4) to establish a standard HRP cell density relationship in order to titrate the extent of lesioned SN against controls. The HRP anatomical tract tracing technique has been discussed above. The methodology pertaining specifically to the current study will be discussed below, followed by the results of the study. These will be described and critically evaluated with comparison to the

literature.

3.2 CONFIRMING THE CROSSED PROJECTION, ESTABLISHING ITS EXTENT AND SITE OF DECUSSATION

The experimental protocol followed in this section was firstly to determine the nature of the crossed mesostriatal projection in naive animals in order to establish a baseline from which to evaluate lesioned animals. Of particular importance was establishing the size of the projection so that its augmentation or attenuation in response to lesions could be measured.

The site of decussation of the crossed projection was also of crucial importance as this was needed for the behavioural study which follows (Chapter 4). Knowledge of where the crossed projection decusses would enable the placement of a lesion within the ventral mesencephalon such that it induced rotation ipsilateral to the lesioned side while sparing the crossed projection.

3.2.1 CONTROLS

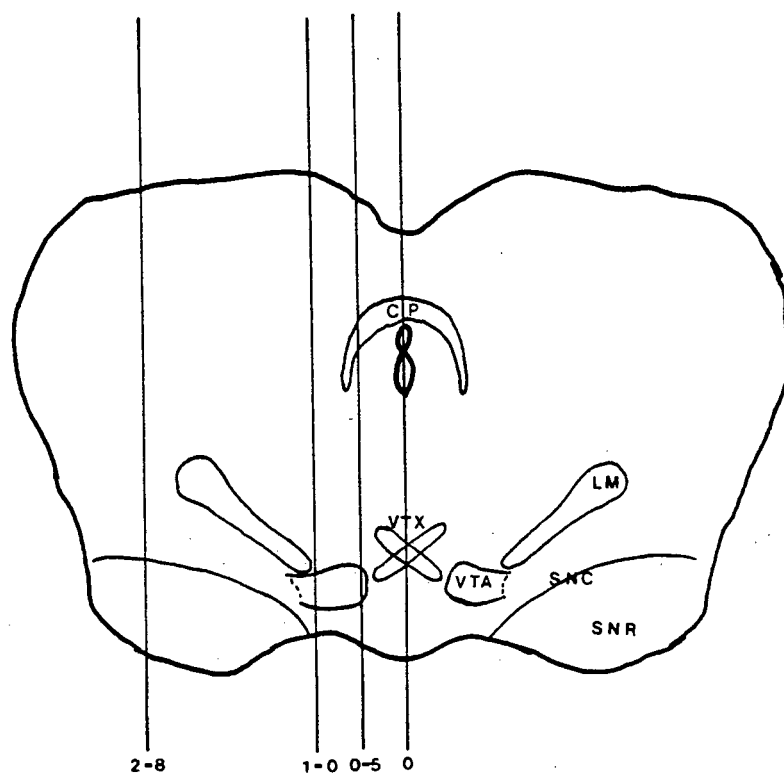
Naive rats were infused with HRP within the caudate nucleus unilaterally. The target co-ordinates for the HRP deposition were maintained consistently at AP=7.8 LAT=2.8 Depth=4.5. This target deposition site resulted in a HRP release zone ventromedially within the rostral striatum. A representative example of the

extent of the deposition site is shown in fig. 3.1B. A survival time of 48 hours was allowed following which the subjects were perfused and histological sections prepared from AP=4.0 to AP=1.0 or 1.8. The HRP histochemistry was carried out and cell counts of the ventral mesencephalon were done according to the schema illustrated in fig. 3.1A. The pattern of HRP labelling of ventral mesencephalic somata is illustrated in fig. 3.2 #C6. and table 3.1. (table of C6). Tables 1 to 11 describing the results of 11 similar experiments in quantitative terms are presented in appendix 3.

FIG 3.1. A The grid schema for HRP labelled cell counts. The cells lying within the coronal sections of mesencephali were counted according to these demarcations using an eyepiece mounted graticule. Measurements are in mm.

FIG 3.1. B A representative coronal section of a striatal HRP deposition site. The target co-ordinates of AP=7.8 LAT=2.8 Depth=4.5 resulted in a ventromedial deposition within the rostral striatum. The black region represents the focus of the release zone centered by the cannula track. The grey region represents the extent of HRP spread.

A



B

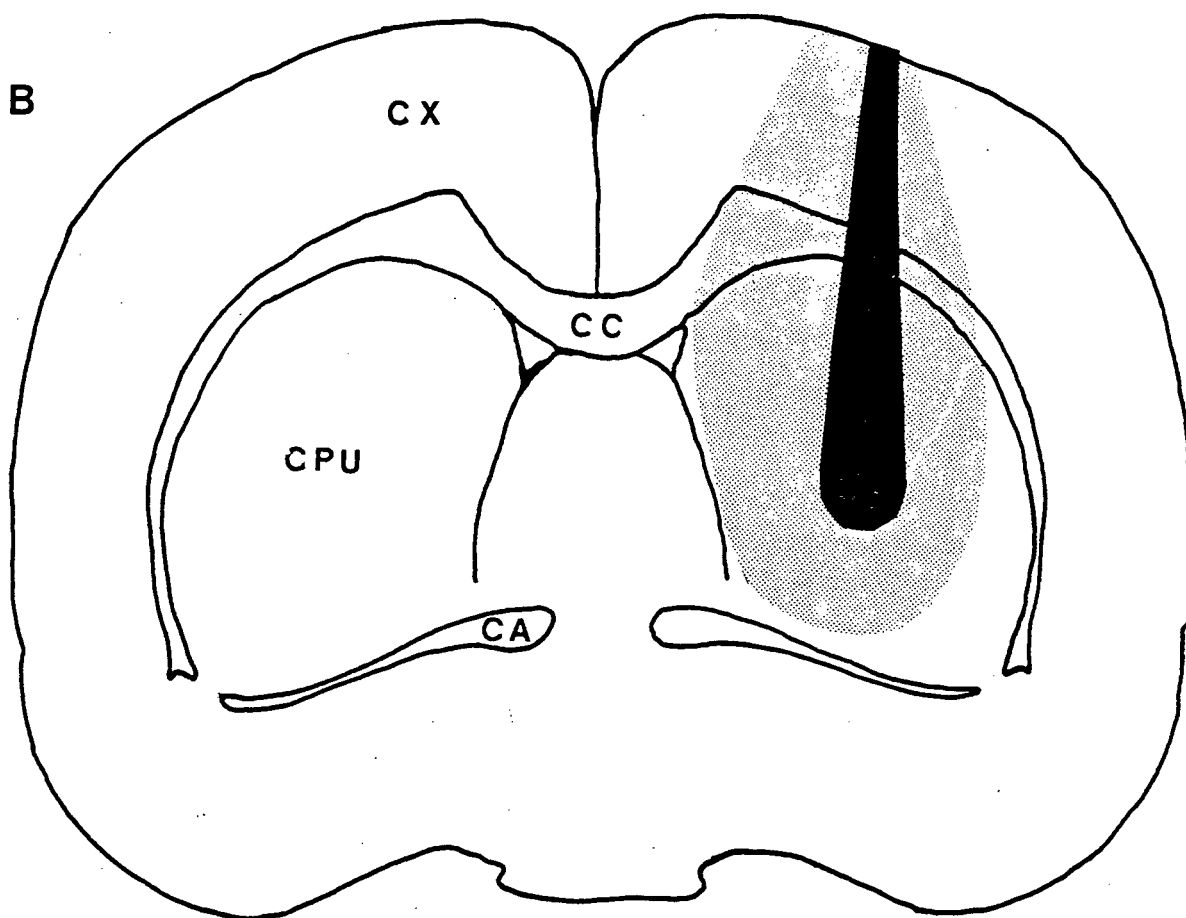


Table 3.1 Rat C6. HRP control group. Cell counts of HRP labelled somata within the ventral mesencephalon between AP=3.6 and AP=2.0. The HRP (2ul) deposition site was restricted to the dorsomedial striatum. HRP halo extending into the corpus callosum and cortex on the ipsilateral side. Dense filling of the striatum extends far caudally (AP=6).

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	1	0	0	0	0	60
3.2	3	6	0	4	12	64	441
3.0	3	6	0	4	12	64	>600
2.8	4	10	12	13	135	427	>600
2.6	3	2	6	13	135	427	>600
2.4	4	8	12	12	123	333	>600
2.2	4	11	7	25	211	268	>600
2.0	3	9	11	41	182	152	>600
TOTAL	28	83	42	126	796	1960	2594

Total ipsilateral cells IL=>6857

Total contralateral cells CL=251

CL/IL*100<3.61

CI=16.1 (Eq 3.1 p142)

FIG 3.2. Representative camera lucida drawings of coronal mesencephalic serial sections between AP=3.4 and 1.6 illustrating the distribution of HRP filled somata following unilateral caudate deposition. Sections conform to the nearest Pelligrino et al. (1979) section. Control animal#C6. Each closed circle represents a HRP filled cell. HRP labelled cells are seen within the SNC and VTA. At AP=3.2 they begin to cross the midline. Most crossed cells are seen between AP=2.6 and AP=1.8, the co-ordinates of the DTV according to Pelligrino et al. (1979). DTV ventral tegmental decussation, IP interpeduncular nucleus, LM medial lemniscus, SNC substantia nigra pars compacta, SNR substantia nigra pars reticulata, VTA ventral tegmental area.

C 6

U

3.4



3.2



3.0



2.8

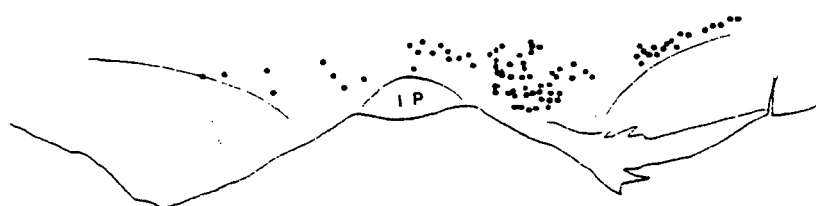
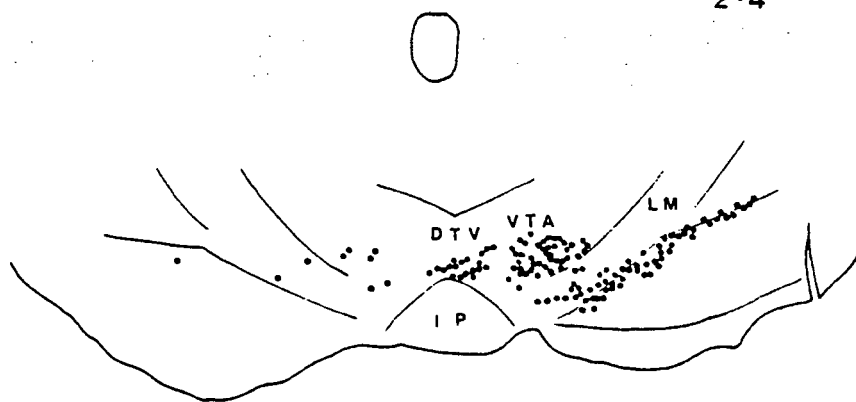


Fig. 3.2 continued.

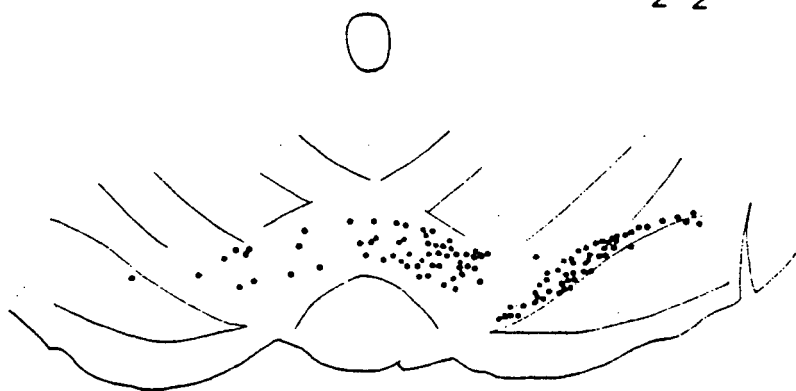
2·6



2·4



2·2



2·0

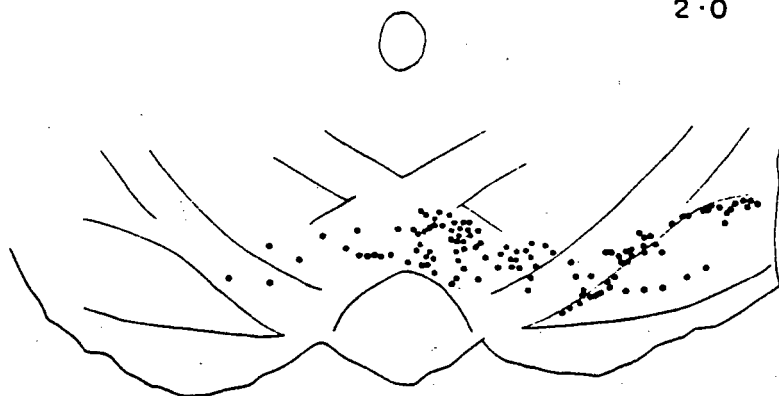
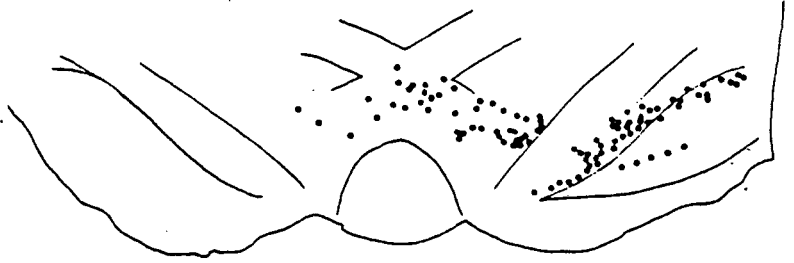


Fig. 3.2 continued.

0

1·8



0

1·6



The intralaminar thalamus was labelled ipsilaterally following unilateral striatal HRP deposition. In particular, the parafascicular thalamus was densely labelled, although the ventroanterior, ventrolateral complex was densely labelled as well. This was expected as thalamostriatal projections from these nuclei have been described (Powell and Cowan 1956, Nauta et al. 1974).

Typically the ventral mesencephalon was labelled ipsilaterally from the anterior extent of the SN (AP=3.8 to 3.6) to the caudal limit of AP=0.8, which included the central linear nucleus, dorsal raphe nuclei, and cells within the decussating fibres of the brachium conjunctivum (BC). The SN labelling could be separated into the SNC cell cap, and a more medial group of cells comprising medial SNC cells and the VTA. There were also some labelled cells within the pars reticulata of SN, particularly more caudally. Between AP=2.6 and AP=1.4 the ipsilateral medial VTA cells formed a diffuse distribution across the midline within the decussating fibres of the DTV. The DTV is primarily a decussation of red nucleus efferents (Kappers et al. 1936) and is the major midline/medial structure in the ventral mesencephalon between AP=2.6 and 1.4 (Pelligrino et al. (1979). Contralaterally, the diffusion of labelled somata within the DTV was maintained, becoming more sparse within the VTA. There were isolated labelled somata within the medial SNC as well.

A HRP density rating of the deposition site was allocated to each

animal. This was established by inspection by an experienced observer and rated between 0 and 10 under blind conditions. Where HRP density was of a high order, 8 to 10, it was difficult to count the ipsilateral cells accurately as cells were often superimposed. This led to an overestimation of the proportion of crossed cells to ipsilateral cells, indicated as such in the tables by >x values for ipsilateral counts. The proportion of contralateral to ipsilateral cells derived from 11 animals ranged from 0.31% to 3.61% with a mean \pm SEM of $1.86 \pm 1.04\%$, table 3.2. The true extent of the crossed projection is thus around 2% of the ipsilateral projection.

Most of the crossed cells within the contralateral ventral mesencephalon were located between the midline and a line 1mm lateral to it. Of three animals with optimal HRP infusions, an average of 233 cells were seen within this region (table 3.3). This region, between AP=2.4 and AP=1.0, contained the DTV and VTA rostrally, and the DTV exclusively more caudally. 82.3% of the crossed cells fell within these two regions. Fewer crossed cells were seen within the SNC, or A9 region (49.6 cells). Since the somata of the crossed projection are widely distributed in the ventral mesencephalon, it has been named the crossed mesostriatal projection rather than crossed nigrostriatal projection.

For the purposes of comparison with experimental groups with manipulations of the crossed mesostriatal projection, a contralateral index (CI) was calculated. The rationale for this index is presented in the next section. The CI for control animals

varied from 1.9 to 16.1, with a mean \pm SEM of 5.8 ± 4 (table 3.2).

3.2.2 NORMALISATION RATIONALE, ESTABLISHING A STANDARD HRP DENSITY, CELL COUNT CURVE.

Cell counts were normalised to take into account variations in numbers of sections and HRP density within the ipsilateral SN. The density factor would vary with the amount of HRP deposited in the striatum, and the degree of uptake. Uptake would in turn be subject to inter-animal variation, as well as differences in the HRP sample used. These spurious differences would complicate the comparison between individual animals and blur real differences between experimental and control animals caused by experimental manipulations like lesions of specific anatomical regions. Thus, in order to justify weighting equations, the relationship between HRP density and ventral mesencephalic cell counts was established.

The data from the control animals described above was normalised for the number of sections and is presented in table 3.2 below. Fig. 3.3 illustrates the ipsilateral cell counts from 16 animals plotted as a function of the ipsilateral density rating on a scale of 0 to 10. Ipsilateral cell counts varied from 1110 to 6857. HRP density varied from 4 to 10. The linear regression through 0 is $y=569*x+-1666$ DF=14 $t=0.201$ $P=0.855$; while the Spearman rank test yields a value of $\rho=0.786$, significant at the 0.01 level. This justifies the use of a normalising function based on HRP density.

Based on a significant linear relationship between cell counts and ipsilateral HRP density, two normalisation equations were

designed. In order to assess contralateral labelling and facilitate inter-animal comparison, a contralateral index (CI) was calculated.

$$CI = (1000 * CL) / (um * n * ILR) \quad (3.1)$$

CL=contralateral labelled cell counts

um=nominal section thickness

n=number of sections

ILR=ipsilateral density rating

In order to assess the extent of ipsilateral depletion of SN cells induced by 6-OHDA, retrograde labelling of the lesioned SNC using HRP was done. A lesion severity index, or percent depletion (%D) was derived in order to facilitate inter-animal comparison. This index, %D, was the inverse of the normalised ipsilateral cell counts, lesioned to control. Normalisation incorporated compensation for differences in section number, and HRP density. The standardised ipsilateral SN cell count used as the reference against which to measure the lesioned SN cell count was 6847.8; the average of 3 animals with maximal uptake and ipsilateral labelling (table 3.3). This cell count was the summed total of 36 50um sections between AP=3.6 and AP=1.8.

$$\%D = [1 - [(IC/6847.8) * (36/n) * (10/hrp)]] \times 100 \quad (3.2)$$

IC=Ipsilateral cell counts

6847.8=standardised control ipsilateral cell count (see above)

n=number of sections observed

36=number of 50um sections between AP=3.6 and AP=1.8

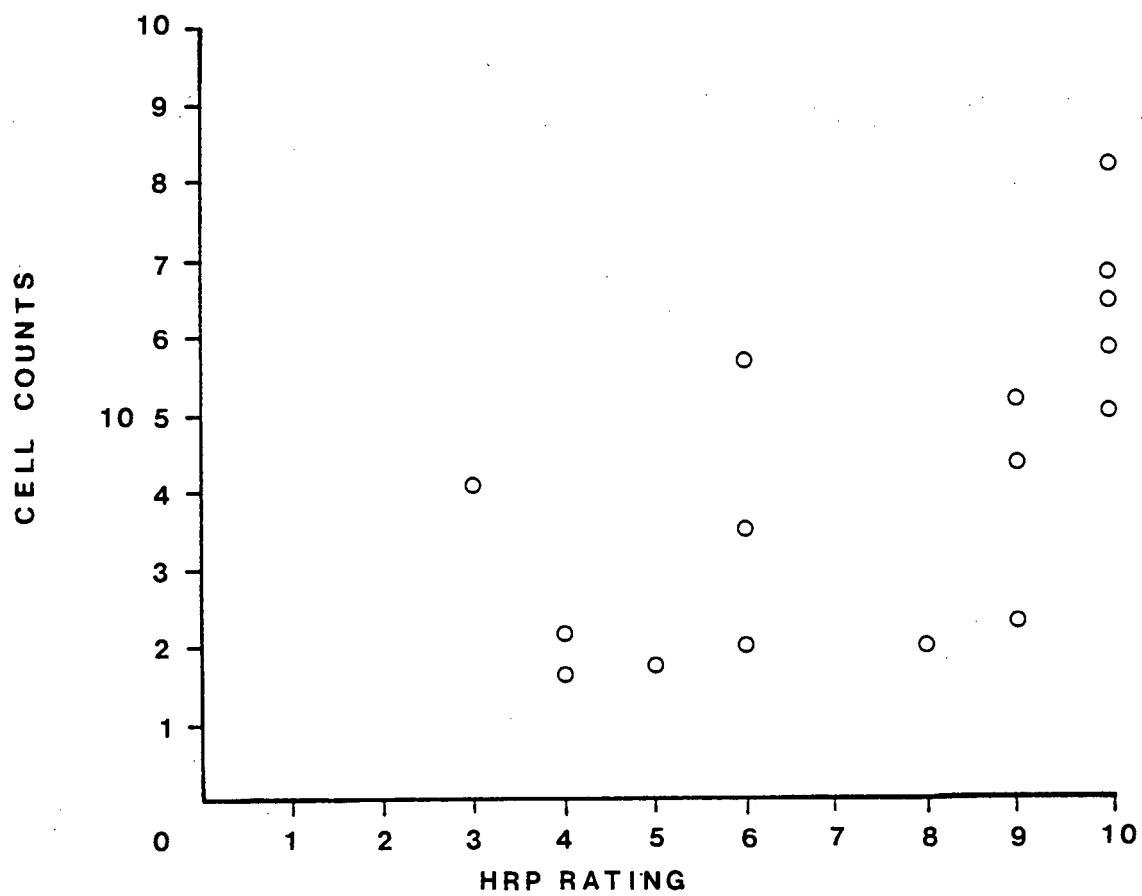
10=maximal ~~HRP~~ density rating

hrp=actual ~~HRP~~ density

Table 3.2 Summary of control data presented above. CL=contralateral cell counts, ILC=ipsilateral cell counts corrected for number of sections, ILR=*ipsilateral* HRP density rating, CI=contralateral index.

RAT	SECTIONS	UM	CL	ILC	ILR	CL/IL*100	CI
27	7	100	9	1110	4	0.8	2.57
29	15	100	56	1901	6	2.9	5.33
30	4	100	6	1900	4	0.31	3.00
33	7	100	41	1984	8	2.0	6.51
34	10	100	79	2584	9	3.0	7.90
37	7	100	8	1266	5	0.63	1.90
C1	32	50	70	5000	10	1.4	4.38
C2	25	50	67	5120	9	1.3	2.00
C5	32	50	172	6000	10	2.86	9.77
C6	28	50	248	6857	10	3.61	16.10
C8	34	50	80	4706	9	1.69	5.20
Mean						1.86	5.8
+-SEM						1.04	4

FIG 3.3. Graph of HRP deposition site density vs labelled cell counts. Significant at the 0.01 level, Spearman rank test.



$$Y = 569 X \pm 1666$$

Table 3.3 Summary cell counts of ^{rats}C1, C5, and C6 corrected for number of sections and presented as labelled cells per 200um. The animals were chosen on the basis that they had complete cell counts with maximal density, thus negating the need for normalisation.

AP	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	0	0	0	4	2.5
3.4	0.8	0	0.8	2.8	19.2	388.8
3.2	0.7	0	0.2	1.2	6.5	>346
3.0	12.3	3.7	6.2	29.3	207	>400
2.8	4	5.5	7.5	50	278	>550
2.6	3.5	3.5	8	58	335	>400
2.4	5.2	5.6	8	110.5	341	>500
2.2	5.3	15.5	20.6	144	304	>400
2.0	5	13.4	17.4	124.4	151.7	>400
1.8	4.8	9.6	35.2	140	346.4	>605
1.6	3.5	8.6	22.9	107.5	239.5	345
1.4	2.9	6.9	14.9	44.6	201.2	289.5
1.2	0.6	3.5	8	32.6	128.6	125
1.0	0	1.4	6.7	18.7	70.7	84

TOTAL	49.6	77.2	156.4	863.6	2632	>5038
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Total contralateral cells=283.2

Total ipsilateral cells>9534.7

52 sections

>164 ipsilateral labelled cells per section

Summed to AP=2.0 Total ipsilateral cells>5756 32 sections

>180 ipsilateral cells per section

1011005

3

3

3.2.3 EVIDENCE FOR THE SITE OF DECUSSATION OF THE CROSSED MESOSTRIATAL PROJECTION.

The high frequency of crossed cells within the mesencephalon, as well as their paucity within thalamic structures inferred a mesencephalic decussation rather than crossing via thalamic commissures as suggested by Pritzel and Huston (1980). The attenuation of crossed cell counts with 6-OHDA lesions of the SN provided additional evidence for this assertion (Douglas et al. 1984). This evidence, and the greatest occurrence of crossed cells between AP=1.4 and AP=2.4, which corresponds to the location of the DTV, suggested the DTV as the mesencephalic structure bearing the crossed projection.

The following experiment was designed in order to determine unequivocally whether the crossed projection has a thalamic or mesencephalic decussation. Three experimental procedures were followed; 1) thalamic transection, 2) mesencephalic transection, 3) discrete 6-OHDA lesion of the DTV. HRP was then infused into the striatum ipsilateral to the lesion and cell counts of the ipsi- and contralateral ventral mesencephalon were performed. The experimental rationale was that if the crossing had a thalamic trajectory, then thalamic section should disrupt it. It was suspected that this was not the case. The final experiment was designed to disrupt the structure most likely to bear the crossed projection, the DTV.

Midsagittal thalamic transections were performed on 5 animals prior to unilateral caudate HRP infusion. Thalamic transection was performed with a pendulum microknife based on the design of Swadlow and Sneiderman (1969). The knife consisted of a shaft 150mm long. This had a slot 20mm in depth at one end. This end had a fulcrum mounted at the end bridging the slot. The knife itself was 10mm long, soldered to the fulcrum. There was a balance shaft mounted 280mm up the shaft parallel to the plane of the knife swing. There was a shaft from the fulcrum to this balance shaft such that it was in contact with the balance shaft. When the lesion was performed, the fulcrum shaft was moved back and forth against the balance shaft so that its movement was evenly guided. It was lowered under stereotaxic guidance to the target co-ordinates AP=4.8 L=0.0 D=7.0. Lesioning involved moving the knife about its fulcrum subtending an arc of 13 degrees rostral and caudal of normal in the midsagittal plane. Other than the hemispheric disconnection provided by the lesion, the subjects were treated in an identical fashion to controls.

In a further 5 rats midsagittal transection of the mesencephalon was performed using the identical technique as for the thalamic transection. Target co-ordinates for the lesion were AP=2.0 L=0.0 D=8.5.

In 5 animals, discrete 6-OHDA lesions (2ul 0.6%) of the DTV were performed. The lesion was effected by an oblique approach to the midline to avoid damage to the periaqueductal gray and raphe nuclei. This had the added advantage of avoiding the sagittal

suture which is prone to rupture when surgically antagonised. HRP infusion into the ipsilateral caudate of the 6-OHDA lesioned animals followed 24 days post lesion.

In 3 animals, vehicle (0.05% ascorbate) was injected into the DTV to assess the effect of the mechanical disruption produced by the cannula and pressure ejection of vehicle on contralateral labelled somata.

THALAMIC TRANSECTION.

Table 3.4 and fig. 3.5 (#TC1) show the detailed cell counts of a representative thalamic sectioned animal. There was no attenuation of HRP labelled somata within the contralateral hemisphere as compared to controls. The table shows the total ipsi- and contralateral cell counts within the ventral mesencephalon between AP=3.6 and AP=1.8, and the figure the distribution of labelled somata within this region. The contralateral index (CI) for these 5 animals ranged from 3.61 to 14.85 and was statistically unchanged from control levels using the Mann Whitney U test (table 3.5, fig. 3.7D).

The extent of the lesion produced by the thalamic transection as determined by histological examination in a representative animal (TC1) is shown in fig. 3.4B. The rostral and caudal limits of the lesion were the anterior commissure and supramamillary decussation respectively. In one animal (M42) the caudal limit included the supramamillary decussation. Ventrally, the lesion extended into

the third ventricle thus transecting the entire thalamus.

Table 3.4 Cell counts in the ventral mesencephalon between AP=3.6 and AP=1.8 in animal (TC1) with midthalamic section. HRP deposition site restricted to striatum. Slight spillover to ipsilateral corpus callosum.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	31	0	0	0	0	0
3.4	5	>400	7	0	0	0	1
3.2	3	>300	9	1	0	0	0
3.0	4	>400	69	0	2	0	2
2.8	4	>400	100	9	2	2	0
2.6	3	>300	107	9	7	0	2
2.4	5	>500	337	28	20	10	7
2.2	4	>400	280	50	35	8	7
2.0	5	>500	311	108	37	11	8
1.8	3	260	101	30	28	7	4
TOTAL	34	3491	1321	235	131	38	33

Total ipsilateral cells IL=>5047

Total Contralateral cells CL=203

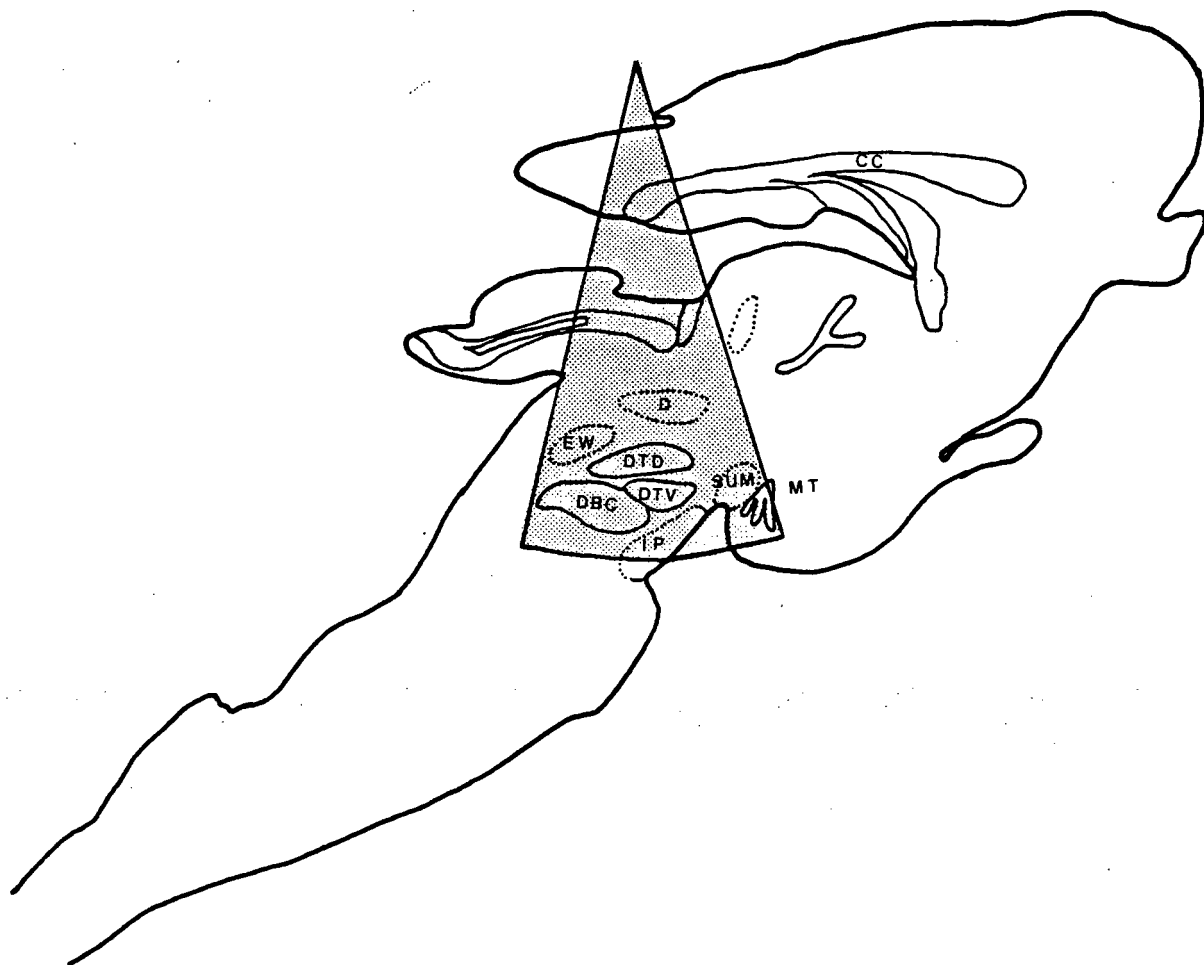
CL/IL*100<4.0

FIG 3.4. Pelligrino et al. (1979) sagittal section 0.1mm illustrating the structures sectioned by the sagittal transection lesion. The grey area represents the extent of the transection. D nucleus of Darkschewitz, EW nucleus of the oculomotor nerve, DTD decussation of the dorsal tegmentum, DTV decussation of the ventral tegmentum, DBC decussation of the brachium conjunctivum, IP interpeduncular nucleus SUM supramamillary nucleus, MT mamillothalamic tract, CC corpus callosum, PV paraventricular nucleus of thalamus, AM anteromedial nucleus of thalamus, CTH thalamic commissure, PVH paraventricular nucleus of the hypothalamus.

A Mesencephalic transection.

B Thalamic transection.

A



B

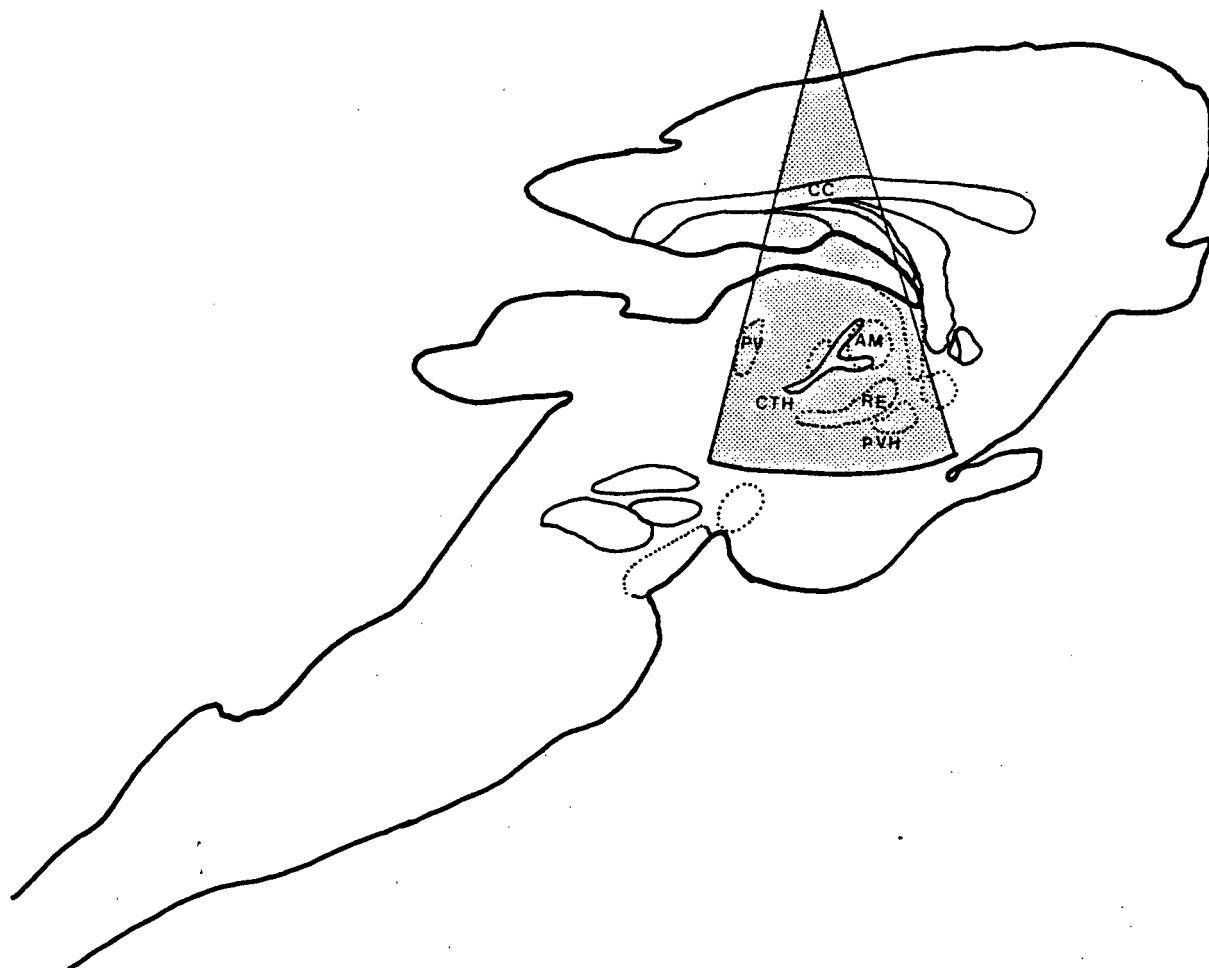
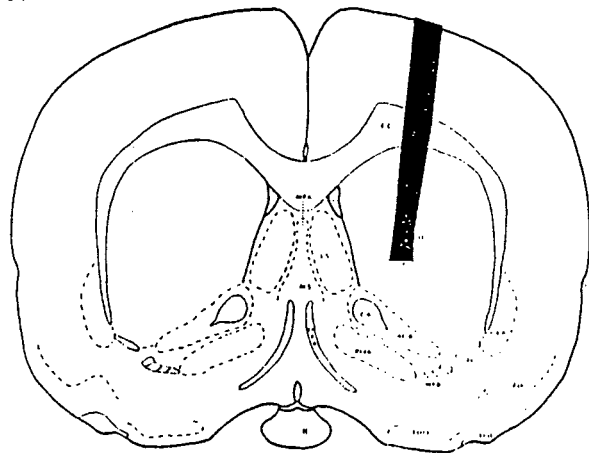
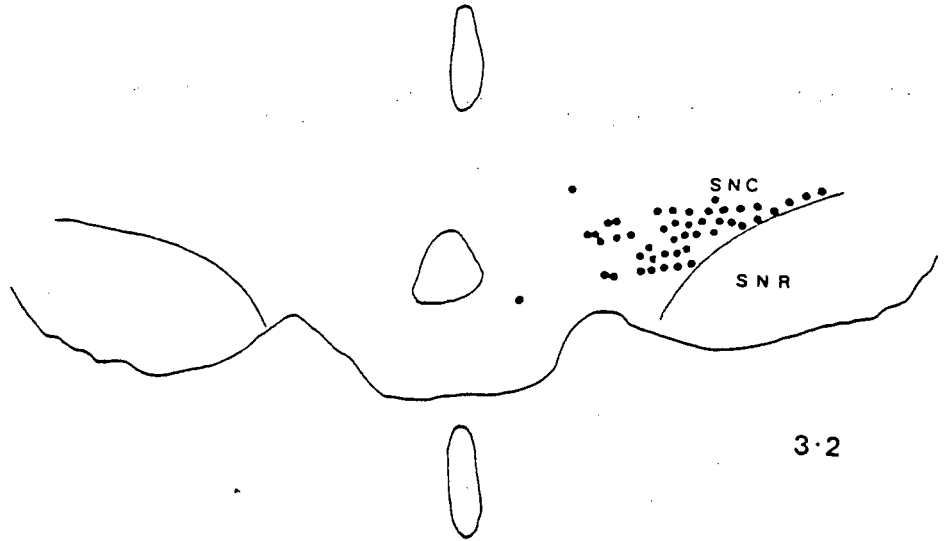


FIG 3.5. The effect of thalamic transection on the HRP labelling of the mesencephalic somata in rat#TC1. Camera lucida drawings conforming to Pelligrino et al. (1979) coronal sections AP=3.4 to 1.6. Each closed circle represents a HRP filled cell. The top diagram illustrates the striatal HRP deposition site. The pattern of labelling is similar to controls, eg C6.

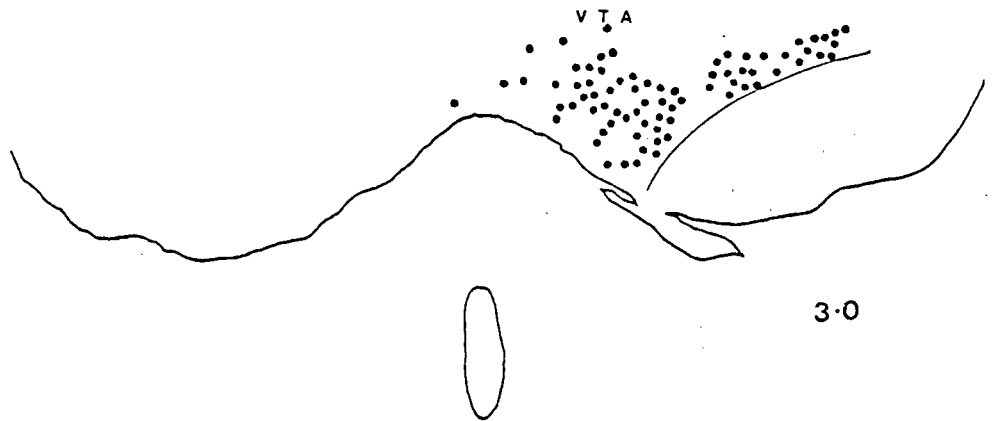


TC 1

3.4



3.2



3.0

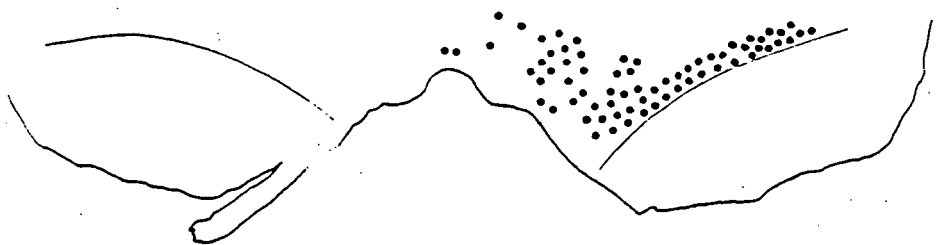
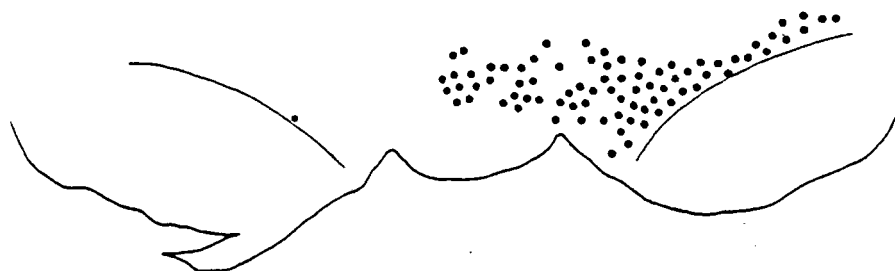
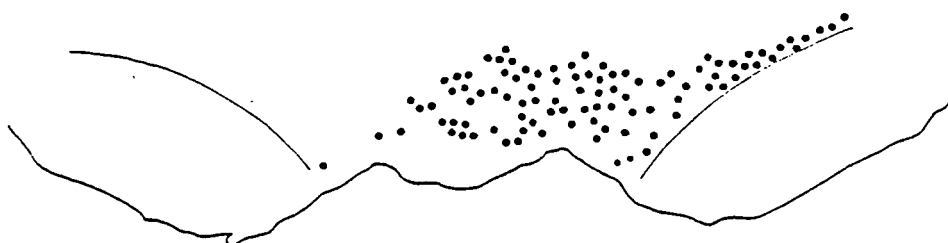
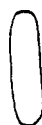


Fig. 3.5 continued.

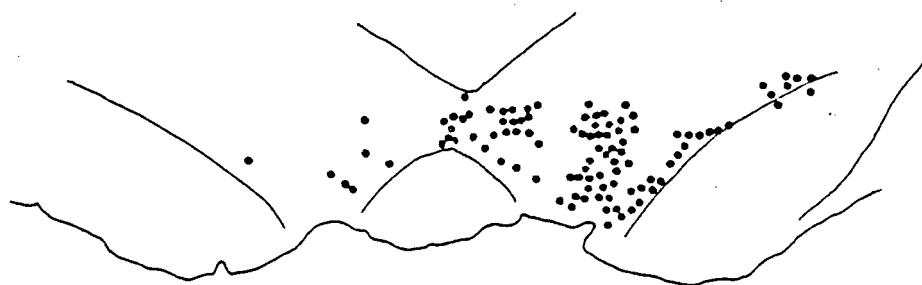
2·8



2·6



2·4



2·2

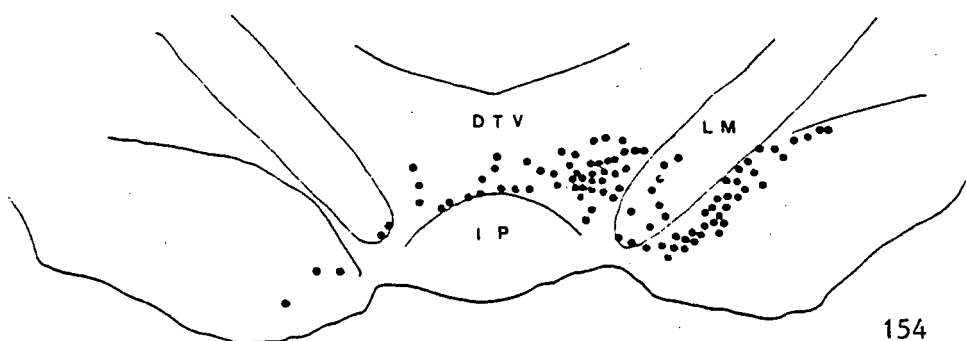
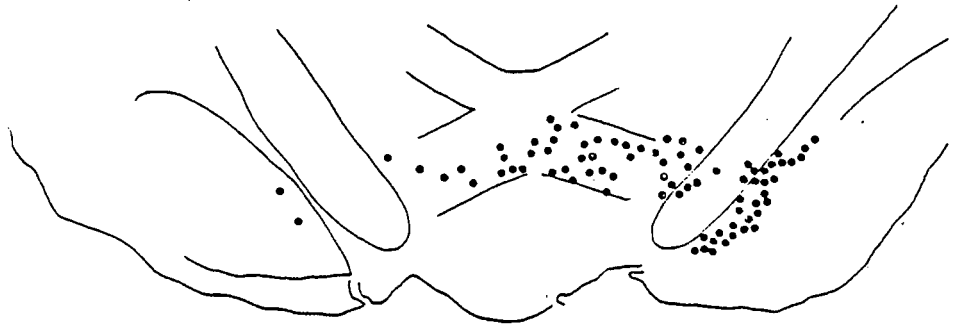


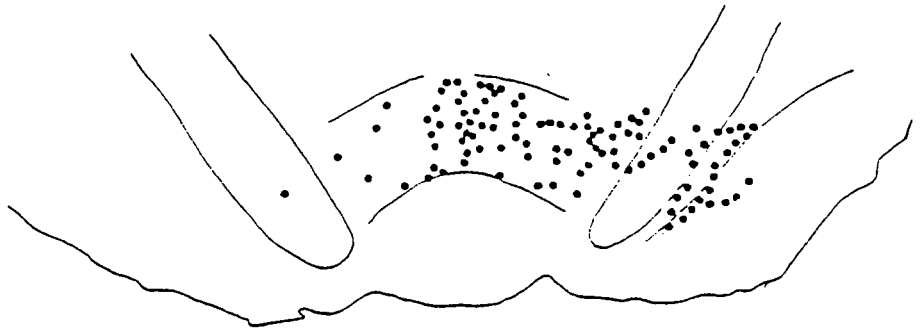
Fig. 3.5 continued.



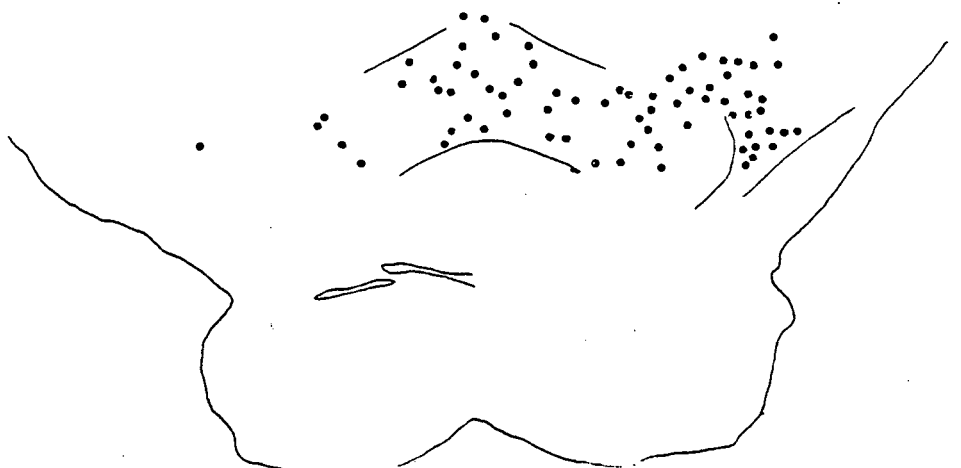
2.0



1.8



1.6



MESENCEPHALIC TRANSECTION

The extent of the mesencephalic transection is illustrated in fig. 3.4A. The lesion involved the ventral tegmental, dorsal tegmental, and supramamillary decussations. The ventral extent included the dorsal aspect of the interpeduncular neurones, while more dorsally the posterior commissure and anterior periaqueductal grey were sectioned. These lesions totally disrupted the crossed projection, reducing CI to 0 (table 3.5, fig. 3.7B).

6-OHDA LESIONS OF THE VENTRAL TEGMENTAL DECUSSATION.

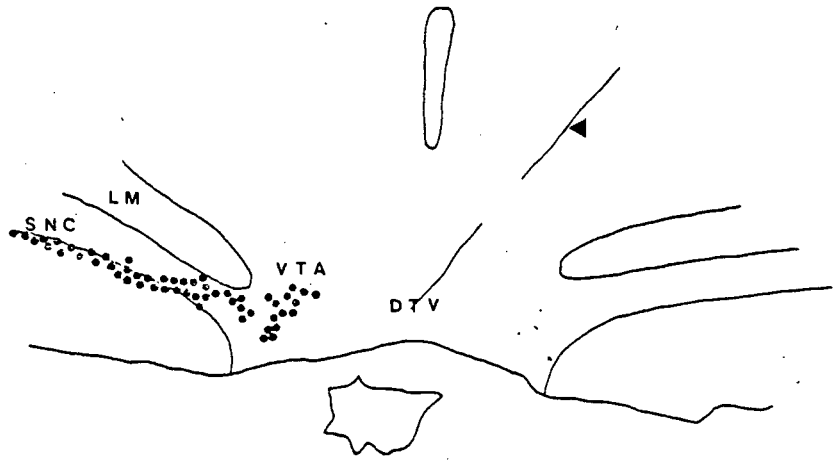
Most of the HRP labelled cells in the contralateral ventral mesencephalon of control animals were found between AP=2.6 and 1.4, and between the midline and a line 1mm lateral to it. These are the co-ordinates of the DTV. In addition, comparison between transections of the thalamus and ventral mesencephalon had shown that the mesencephalic transection disrupted crossed cells while the thalamic transection had no effect. The mesencephalic transection affected structures other than the DTV, however, and discrete 6-OHDA lesions restricted to the DTV were therefore carried out. These lesions disrupted the crossed projection to varying degrees depending on their precise location and extent. One animal with a large lesion of the DTV (233) had total disruption of crossed cells (CI=0), while another with a small lesion located anterior to the DTV in the supramamillary decussation (SMX, 267) showed a distribution of crossed cells

similar to controls $CI=8.36$. Wherever the DTV was affected to some degree by the 6-OHDA lesion there was a marked reduction of crossed cells as evidenced by a significantly reduced CI compared to controls ($CI=0$ to 1.52 , $p<0.001$, Mann Whitney U test, $n=4$). The HRP filled cell distributions for 266, 267, 268, and 269 are illustrated in fig. 3.6. A representative example of the ventral mesencephalic HRP filled cells with a 6-OHDA lesion of the anterior DTV is contrasted with thalamic transection, mesencephalic transection, and a control animal in fig. 3.6. The effect of a 6-OHDA lesion of the DTV on ventral mesencephalic HRP labelled cells is illustrated in photograph 2. Photograph 1 illustrates the pattern of labelling in a representative control animal. Vehicle injections into the DTV had no effect on contralateral labelling, which was similar to controls. CI for vehicle injected animals varied from 5.33 to 8.47 . This indicates the 6-OHDA, and not mechanical damage caused by the cannula or vehicle pressure ejection, was responsible for the attenuation of the crossed projection.

Table 3.5 Summary of HRP labelled somata within the ventral mesencephalon of animals with thalamic transections (TX), mesencephalic transections (MX) and 6-OHDA lesions of the DTV. The 6-OHDA lesion of one animal was within the supramamillary decussation (SMX). DTVC are controls with vehicle infused into the DTV. *um* is the section thickness in μm . CL = contralateral cell count. ILR = ipsilateral HRP density rating. CI = contralateral index.

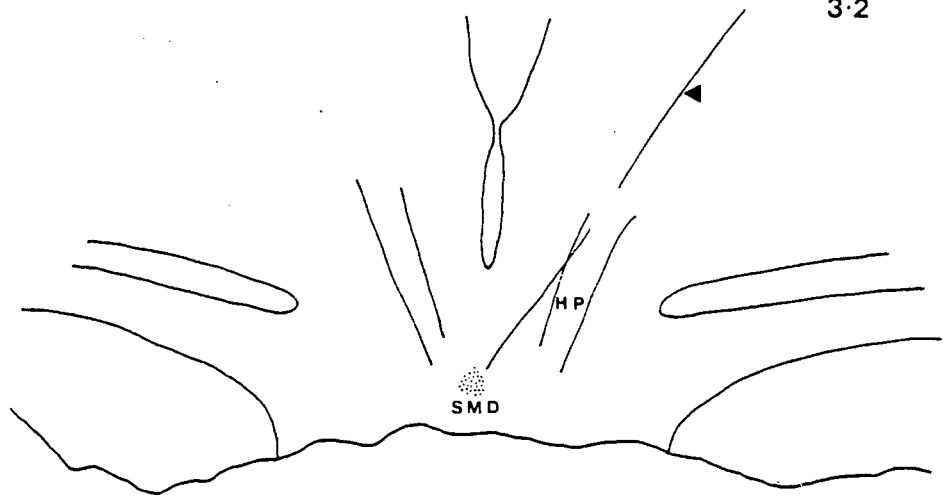
LESION	RAT	SECTIONS	um	CL	ILR	CI
TX	TC1	34	50	202	7	14.85
TX	M3	25	50	14	2	3.73
TX	M4	24	50	19	2	5.28
TX	M42	41	50	37	4	3.61
TX	M43	29	50	34	3	5.86
MX	M7	27	50	0	1	0
MX	M8	27	50	0	2	0
MX	M45	30	50	0	2	0
MX	M128	27	50	0	5	0
MX	M129	13	50	0	4	0
DTV	268	45	50	24	6	1.52
DTV	269	43	50	5	3	0.58
DTV	233	25	50	0	3	0
DTV	266	35	50	8	9	0.51
SMX	267	35	50	117	8	8.36
DTVC	C11	35	50	56	5	5.33
DTVC	C13	31	50	105	7	8.47
DTVC	C14	37	50	151	9	8.16

FIG 3.6. The effect of midline infusion of 6-OHDA employing a 30 degree oblique cannula approach on HRP labelling of mesencephalic somata. Camera lucida drawings of sections showing lesion and a more caudal section if the lesion was very anterior. Ventral mesencephalic cells labelled from striatal deposition sites. Each closed circle represents a HRP filled cell. Arrow shows cannula track. Subjects 266, 267, 268, and 269 are illustrated. Animal 267 had a lesion of the SMD, and showed some contralateral labelling. There is marked attenuation of crossed cells following 6-OHDA lesioning of the DTV in the other animals. DTV ventral tegmental decussation, HP habelulo-interpeduncular tract, LM medial lemniscus, SMD supramamillary decussation, SNC substantia nigra pars compacta, VTA ventral tegmental area.



267

3·2



2·4

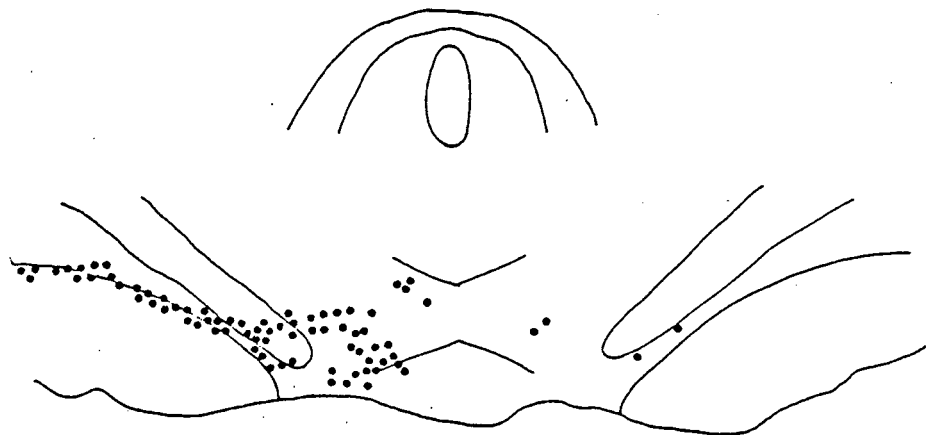
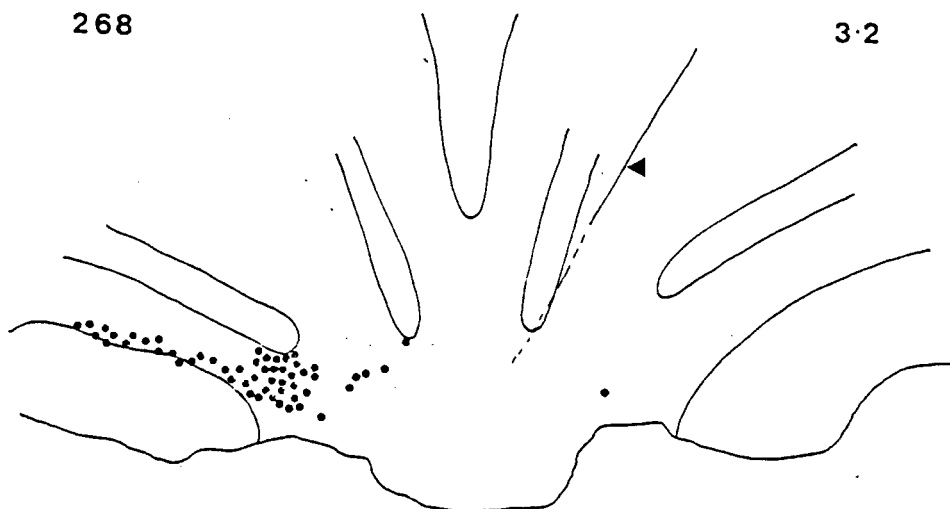


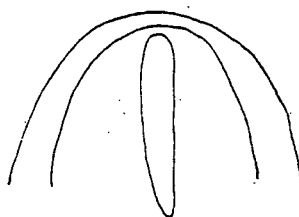
Fig. 3.6 continued.

268

3·2



2·8



269

2·8

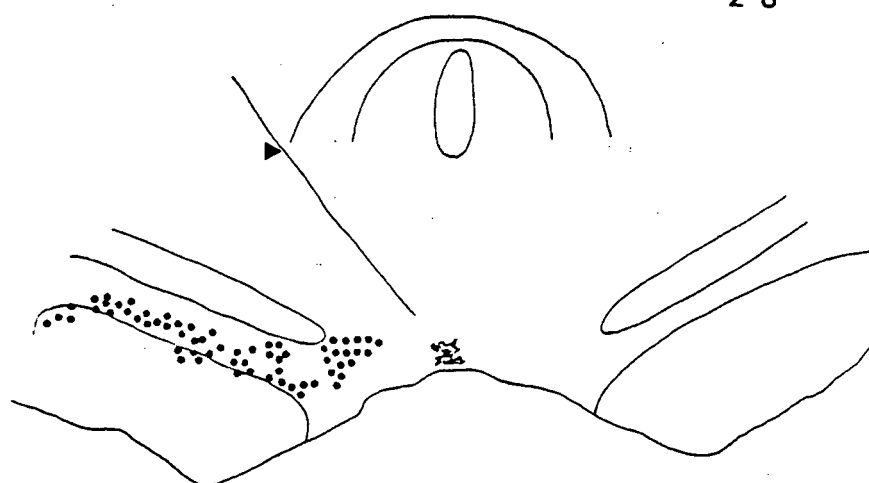


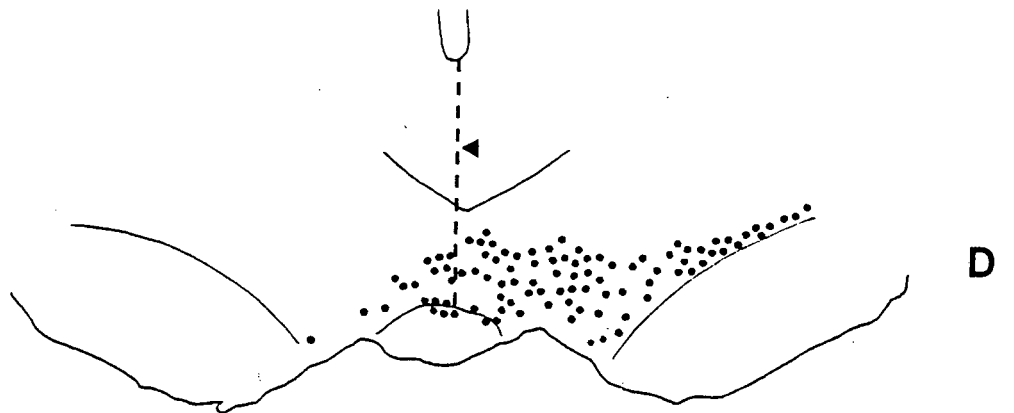
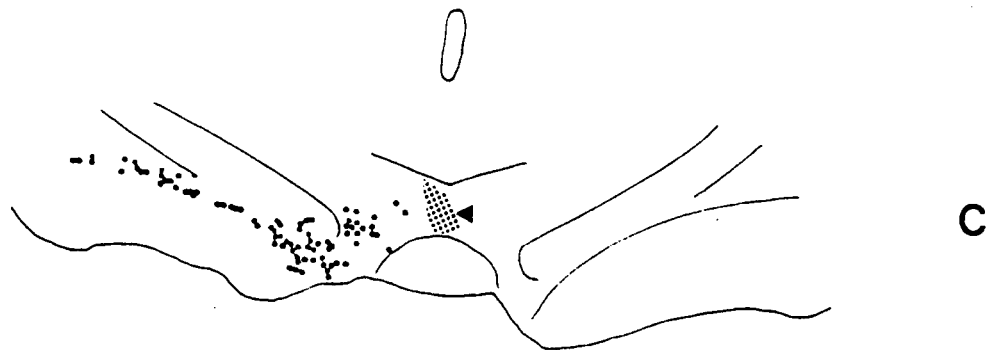
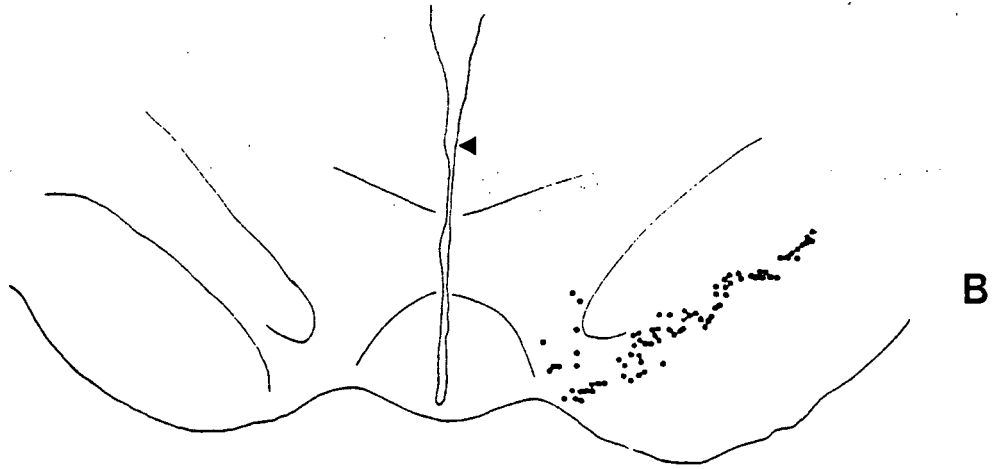
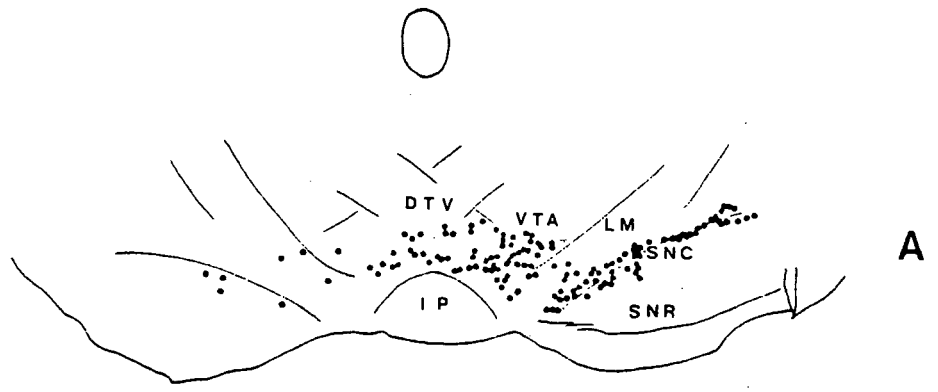
FIG 3.7. A comparison of the effects of mesencephalic transection (B), lesioning with 6-OHDA (C), and thalamic transection (D), on the HRP labelling of somata within the mesencephalon of the rat. In all cases the ventral mesencephalic cells are labelled from striatal deposition sites. Each closed circle represents a HRP labelled cell. Camera lucida drawings conforming to the Pelligrino et al. (1979) section at AP=2.4.

A The top drawing is a control animal.

B Mesencephalic transection prevented the labelling of contralateral cells. The lesion is indicated by the arrow.

C 6-OHDA lesioning of the DTV also attenuated the presence of contralateral cells. The lesion is the grey region marked by an arrow.

D Thalamic transection had no effect on the pattern of mesencephalic labelling. The lesion is anterior to the section shown and thus not directly visible. The dimensions of the lesion are shown by the stippled line.



Photograph 1: Photograph of the ventral mesencephalon of a control animal (C6). HRP filled somata are apparent in the SNC (1), VTA (3) and DTV (4) dorsal to the interpeduncular neurones (5). There are no labelled cells in the ventral SNR though there are a few in the dorsal SNR.⁽²⁾ These might be considered displaced SNC cells. The HRP labelled cells spill over the midline into the contralateral hemisphere, but are mainly located medial to the SNC. The arrow indicates a HRP filled cell in the contralateral VTA.

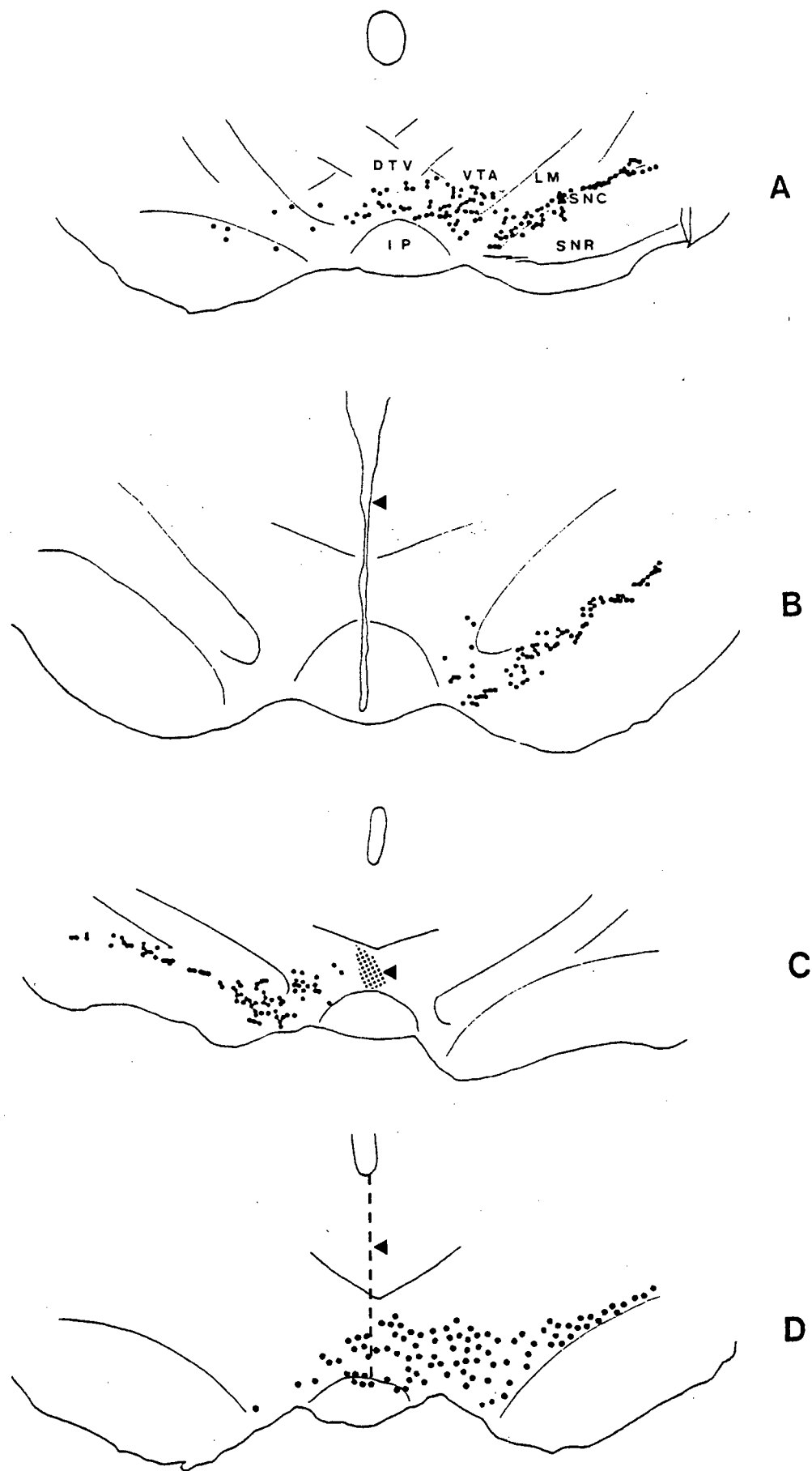
FIG 3.7. A comparison of the effects of mesencephalic transection (B), lesioning with 6-OHDA (C), and thalamic transection (D), on the HRP labelling of somata within the mesencephalon of the rat. In all cases the ventral mesencephalic cells are labelled from striatal deposition sites. Each closed circle represents a HRP labelled cell. Camera lucida drawings conforming to the Pelligrino et al. (1979) section at AP=2.4.

A The top drawing is a control animal.

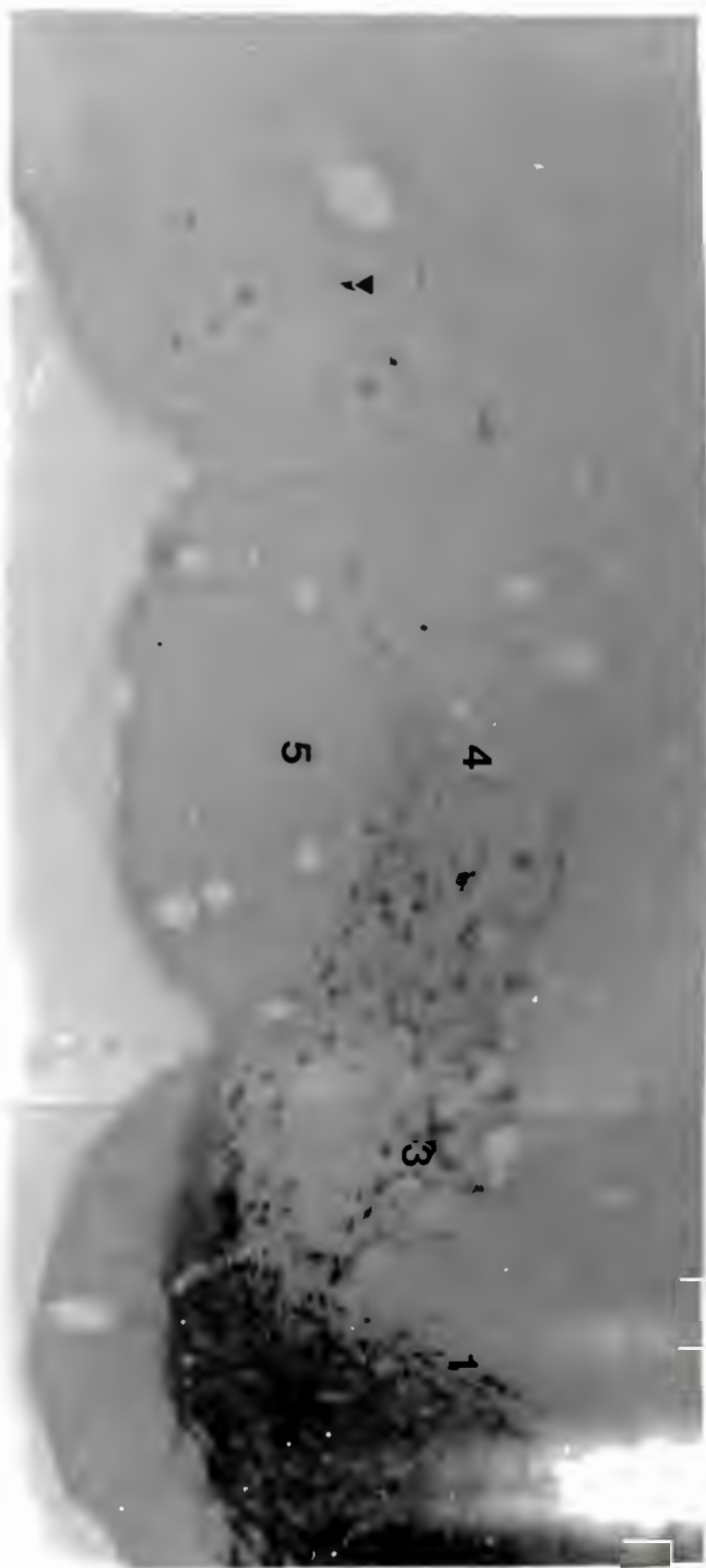
B Mesencephalic transection prevented the labelling of contralateral cells. The lesion is indicated by the arrow.

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D Thalamic transection had no effect on the pattern of mesencephalic labelling. The lesion is anterior to the section shown and thus not directly visible. The dimensions of the lesion are shown by the stippled line.



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Photograph 2: Photograph of the ventral mesencephalon of a DTV lesioned animal. The cannula track is apparent as a diagonally sloped line (arrow). There are HRP labelled somata within the ipsilateral SNC (1) and VTA (3). The contralateral labelling has been totally curtailed. DTV (4), SNR (2).



3.3 DISCUSSION AND CONCLUSIONS

The crossed mesostriatal projection was first described in cats by Royce (1978) and in rats by Fass and Butcher (1981). These workers provide data that added a crossed component to the exclusively ipsilateral NS projection, and raised possibilities for interhemispheric communication of the bilateral nigrostriatal systems. This crossed projection should be termed the crossed mesostriatal projection rather than nigrostriatal, as it arises from ventral mesencephalic structures other than exclusively the SNC. Fass and Butcher (1981) reported the greatest concentration of ipsilateral cells labelled with the retrograde tracer Evans blue (EB) in the rostral SN. The results of the present study suggest that ipsilateral density is maximal about halfway caudally in the ventral mesencephalon. More labelled cells were seen in SNR at caudal levels than rostrally, in agreement with Fass and Butcher. These SNR labelled cells correspond to the Dahlstrom and Fuxe A8 cell group.

The density of the ipsilateral labelling varies from 105 granular blue (GB) labelled somata per 30um section (Altar et al. 1983) to 20-30 GB labelled somata per 20um section (Fallon et al. 1983), and 180-164 HRP labelled cells per 50um section sampled from AP=3.6 to 1.0 and 1.8 respectively (Douglas et al. 1987 in press). The discrepancy between the GB studies is rather surprising, as both Altar et al. (1983) and Fallon et al. (1983) used 5% suspensions of GB processed according to the method of Kuypers et

al. (1977). In particular 105 cells per 30um section implies a total ipsilateral cell count of 9100 cells for the rostrocaudal length of the SN/VTA. This is in general agreement with the ipsilateral values obtained by the HRP method used in this study, 8475 labelled cells, though the total cell count for the Fallon study of 3250 cells for the same thickness of brain is rather low.

Fass and Butcher (1981) reported 2 to 14 EB labelled somata per contralateral SN, mainly within the SNC, but with occasional cells in the SNR. Some cells were seen in the contralateral VTA. Fallon et al. (1983) stated that the crossed cells lay within a sector of the SN/VTA which corresponded to the centre of the ipsilaterally projecting cluster of SN/VTA neurones. The results described in this study are in agreement with the literature, though de-emphasis is placed on SNC labelled cells as very few of these were seen. Most cells described as originating the crossed projection were seen in the DTV and VTA.

There is good agreement between various studies on the proportion the crossed projection forms of the ipsilateral one. Altar et al. (1983) carried out detailed cell counts and reported that the crossed projection was 0.9% to 3.4% of total cell numbers (ie. a somewhat higher percentage of the ipsilateral projection, in good agreement with the ratios of 0.39% to 3.61% of the ipsilateral projection obtained with our study). Thus, it seems clear that the crossed projection is approximately 2% of the ipsilateral projection. This proportion was found to be invariant as a

function of age (Altar et al. 1983), thus there is no developmental proliferation of the crossed projection.

The neurochemistry of the crossed projection has been suggested to be catecholaminergic. Altar et al. (1983) showed a correspondence between retrogradely labelled somata of the crossed projection and catecholamine histofluorescence. In 4 brains processed for simultaneous visualisation of the retrogradely transported dye propidium iodide and catecholamine histofluorescence, all labelled cells also displayed catecholamine histofluorescence. In a comprehensive neurochemical analysis of the crossed projection, using combinations of retrograde fluorescent tracers and transmitter specific histochemistry (immunofluorescence techniques), it was shown that the crossed projection was 95% catecholaminergic. In addition, 50% of the contralateral cells were double labelled for cholecystokinin octapeptide (CCK) as well as the retrograde tracer. Although no direct evidence was presented, the results implicitly suggest that a subpopulation of the crossed projection cells contain both DA and CCK. The findings presented in the present study concur with the crossed projection having a catecholaminergic neurochemistry as it was disrupted by 6-OHDA lesions both ipsilaterally and at its site of decussation. While it was found that there were 6-OHDA resistant cells within the VTA, substantial 6-OHDA lesions of the ipsilateral NS projection abolished contralateral cells. This suggests that the non-DAergic cells of the VTA may not be crossed, though the matter is by no means final.

The crossed projection decussates exclusively via the DTV, as transection or 6-OHDA lesioning of the DTV abolishes crossed cells. Vehicle injection into the DTV had no effect on contralaterally labelled somata. This result allows the design of experiments to test the functional significance of the crossed projection by precisely disrupting or augmenting its nature. As the crossed projection is largely catecholaminergic and a focal lesion of the DTV severely attenuates it, such lesioned animals would be a suitable paradigm.

The crossed projection has been functionally implicated in neuronal plasticity following unilateral NS injury and reciprocal control of the ipsilateral NS systems. Pritzel et al. (1983) suggested that the crossed projection undergoes reactive synaptogenesis following unilateral NS lesioning with 6-OHDA or kainic acid. While the presence of the crossed projection does suggest that it may subserve a contralateral component of neuronal plasticity following injury, its small proportion does cast doubt on the extent of such compensation. This conjecture will be examined in detail in the next section.

The interdependence of the NS systems in the form of reciprocal [DA] regulation bilaterally in the SN and caudate-putamen (CPU) is another possible sphere of crossed projection influence. Nieoullon et al. (1977), using push-pull striatal cannulae, reported that any nigral manipulation decreasing ipsilateral CPU DA release, eg. electrolesion, DA, or amphetamine microinjection, resulted in the opposite effect in the contralateral CPU. Nigral manipulations which increased ipsilateral DA release, eg. potassium

microinjection, resulted in a decrease of DA release in the contralateral CPU. In addition it was observed that spontaneous fluctuations of CPU [DA] followed reciprocal trends 76% of the time. A DTV lesioned animal would make a suitable preparation with which to investigate the significance of the crossed projection in this phenomenon, though the results would probably be negative for the following reasons. Firstly, there is the argument that the crossed projection is too limited to have a significant influence on the contralateral striatum. Secondly, even if this were not the case, a decrease in the neuronal firing rate of the ipsilateral projection by gross ipsilateral SN manipulation would produce a parallel decrease in crossed projection firing. This would induce a reciprocal effect on the contralateral NS system only if the crossed projection affected the contralateral SN. While co-laterals of the crossed projection have not been described, they are a necessary component of a reciprocal control machine. The crossed projection illuminated by retrograde tracers from the contralateral CPU does not have the character to effect the [DA] changes observed in practice. Alternatively, the crossed projection cells would have to terminate in a functionally reciprocal way in the striatum.

A third possible site of crossed projection influence is suggested by the functional character of the brain region housing the crossed projection. Most crossed cells were found within a line 1mm from and including the midline. This mediocentral clustering of cells under the crossed mesostriatal projection banner is strongly reminiscent of the old Dahlstrom and Fuxe (1964)

classificatory system. The A10 or mesolimbic projection was defined as being the region of cells with catecholamine histofluorescence in the basal medial area mainly dorsal to the interpeduncular neurones. They maintained that the cells between the medial SN and the midline were not part of the nigro-neostriatal system, but rather projected to the limbic system. They based this evaluation on the termination sites of the cell somata in question. It is striking that cells of the crossed projection conform almost exactly to the boundary demarcations of the A10 projection. If the crossed projection has any function, it is suggested that this may most likely be in limbic striatal processing, perhaps reciprocal control of [DA] within the ventral or limbic striatum or amygdala.

The findings of our study contradict the finding of Pritzel et al. (1983) that increased labelling of contralateral SNC somata occurred after unilateral SN lesions. Our studies have shown that unilateral SNC lesions (5ul, 10ug 6-OHDA) disrupt both the ipsilateral and crossed mesostriatal pathways (Douglas et al. 1984). The difference between our findings and those of Pritzel et al. (1983) may be explained by the lesion locations of each group. Our lesions were placed anteromedially (Pelligrino et al. 1983; AP=3.0, LAT=1.0), at the origin of the MFB, and as such were well placed to disrupt the crossed element which had decussated more caudally. The lesions employed by Pritzel et al. (1983) were more caudally and laterally placed, at approximately Pelligrino et al. (1979) AP=2.2 LAT=2.5, in the centre of the SN. These lesions may have spared the crossed projection due to their lateral

displacement from the DTV, and their caudal displacement from the MFB.

The findings of the HRP investigation of the crossed mesostriatal projection are summarised below.

1) The crossed mesostriatal projection decussates exclusively in the DTV. There is no additional crossing via thalamic commissures. There is no crossing via the corpus callosum (Fass and Butcher 1981). The effect of DTV lesioning with 6-OHDA and DTV transection confirms the DTV as the site of decussation. The susceptibility of the crossed projection to the neurotoxin 6-OHDA suggests that it is largely catecholaminergic.

2) It does not cross as a well defined fibre tract. There is a cellular dispersion from the ipsilateral VTA through the DTV into the contralateral DTV and SNC. There are also labelled cells anterior to AP=2.6, the rostral boundary of the DTV, although these are a small proportion of the total number of crossed cells. Most crossed cells are found in association with the DTV.

3) The DTV forms the medio-caudal part of the A10 projection, thus crossed cell phenomena may be functionally involved in limbic striatal processing.

4) The crossed mesostriatal projection is approximately 2% of the ipsilateral projection.

LESIONS OF THE VENTRAL MESENCEPHALIC DOPAMINERGIC

CELL GROUPS.

4.1 INTRODUCTION.

4.2 MATERIALS AND METHODS.

4.3 RESULTS.

4.3.1 STANDARD AND RESTRICTED 6-OHDA LESIONS OF THE SN.

4.3.2 THE EFFECT OF STANDARD LESIONS OF THE SN
ON CIRCLING BEHAVIOUR.

4.3.3 RESTRICTED 6-OHDA LESIONS OF THE LATERAL ASPECT
OF SN.

4.3.4 RESTRICTED 6-OHDA LESIONS OF THE VENTRAL TEGMENTAL
DECUSSATION.

4.3.5 RESTRICTED 6-OHDA LESIONS OF THE VENTRAL SN.

4.3.6 RESTRICTED 6-OHDA LESIONS OF THE ANTEROMEDIAL SN.

4.3.7 INVESTIGATION OF THE CROSSED MESOSTRIATAL
PROJECTION AS A FUNCTION OF RECOVERY
FROM MOTOR ASYMMETRY.

4.4 DISCUSSION AND CONCLUSIONS.

4.1 INTRODUCTION

The use of the neurotoxin 6-OHDA to lesion the DAergic cells of the SN is an established method of inducing unilateral striatal DA depletion and thus causing rotational behaviour. The standard procedure used to effect a rotation model in rats is the injection of 4-5ul 0.2% 6-OHDA into the rostral SN, MFB, or lateral hypothalamus (Ungerstedt 1971). More recently it has been suggested that partial lesions of the NS system may be a more useful model of DA depletion disorders like Parkinson's disease (Hefti et al. 1980). The extent of these lesions has been evaluated by the Fink-Heimer catecholamine histofluorescence assay (Dahlstrom and Fuxe 1964; Ungerstedt 1971), HPLC determination of the extent of striatal DA, DOPAC and/or tyrosine hydroxylase depletion (Hefti et al. 1980), and inspection of histologic sections of the lesion site.

In this study, light microscope examination of cresyl fast violet stained sections of lesion sites has been used in order to assess the extent of SN damage. Furthermore, in some cases HRP has been used to evaluate the condition of lesioned SN' by its retrograde transport from striatal deposition sites. It has been shown that evaluation of lesioned SN' with retrograde tracers reflects the degree of striatal DA depletion to within a few percent (Altar et al. 1983).

The effect on the extent and specificity of action of standard

(5ul, 10ug 6-OHDA) compared to restricted (2ul, 4ug 6-OHDA) lesions of the SN is illustrated and contrasted (fig. 4.1). The behavioural effect of standard lesions of the SN is illustrated, and the effects of amphetamine and apomorphine on circling are confirmed. We have shown that the crossed projection can be damaged by standard lesions (Douglas et al. 1984). Partial lesions of the ventral mesencephalon were used in order to induce ipsilateral rotation while sparing the crossed projection. Low doses of 6-OHDA were injected into various ventral mesencephalic structures, using the procedure described in chapter 2. The effects of these lesions on circling behaviour are reported in this section. Crossed projection relevance to recovery from motor asymmetry as shown by circling behaviour was investigated by retrograde labelling of the ventral mesencephalon with HRP. Recovery from sensory-motor asymmetry was also investigated using neurological testing.

4.2 MATERIALS AND METHODS.

Subjects

Fifty-nine male Long Evans hooded rats were used in the study. They were housed under a 12 hour light/dark regime with ad libitum food and water. Groups of 8 rats per cage were maintained after the initial selection based on weight (270-350g) at the start of an experiment.

Behavioural assessment.

Rotational behaviour was monitored by means of a rotometer and BBC microcomputer, as detailed in chapter 2. Furthermore, neurological tests were performed on 16 subjects in order to evaluate their sensory-motor status. These tests are described in chapter 2. They were performed 12 and 72 hours post lesion, and 1 hour before each measurement of rotational behaviour. The test summaries are presented in appendix 4.

Surgery.

Surgery was performed as described in chapter 2. For the standard lesions (n=9), the target co-ordinates were AP=3.0 LAT=2.1 D=8.4; restricted anterolateral SNC (n=12) AP=3.0 LAT=2.8 D=7.2; restricted ventral SN (n=8) AP=2.4 LAT=1.5 ,D=9.6; restricted anteromedial SNC (n=32) AP=3.0 LAT=1.0 D=7.4; and restricted DTV (n=7) AP=2.4 LAT=0.0 D=8.2. 6-OHDA dihydrobromide was administered in solution with ascorbate (2mg/ml, 0.2mg/ml). Standard lesions were 5ul (10ug 6-OHDA), restricted lesions 2ul (4ug 6-OHDA). Subjects were housed together post lesion.

Experimental protocol.

Screening of subjects for hemispherical dominance for rotation direction was carried out on two days prior to lesioning. Animals were placed in the rotometer for 30 minutes [92 to 253] or 60 minutes [254 to 277] and spontaneous circling was continuously monitored. Amphetamine (1mg/Kg, i.p.) was administered thereafter

and behavioural monitoring continued for a further 70 minutes. The dominant hemisphere was subsequently lesioned (see method, chapter 2), and behaviour monitored both spontaneously and in response to amphetamine (1mg/Kg) on days 7, 14, 24 and 32 post lesion. Deciding on dominance was not simple. It was found that the hemisphere dominant for rotation can vary for spontaneous and amphetamine driven behaviour. 46.9% of animals monitored showed opposite rotation directions for spontaneous and amphetamine driven behaviour. If dominance is interpreted as requiring unequivocal agreement between spontaneous and amphetamine driven behaviour, then these 46.9% would have to remain unclassified. Rather than dropping them from the experimental protocol, the amphetamine value was chosen as the deciding one. This was because the higher rotation numbers produced by amphetamine were considered more reliable than the lower scores for spontaneous rotations.

Histology.

After 32 days animals were sacrificed and histology was performed on the fixed brains (see method, chapter 2). Certain subjects (254-277) received striatal HRP injections ipsilateral to the lesion after completion of the behavioural studies. A detailed evaluation of the lesioned SN was made and HRP filled cells were counted on every section between AP=3.6-3.8 and AP=1.8-2.0. In addition camera lucida drawings were constructed of the section conforming to AP=3.8 to 1.8 (Pelligrino et al. 1979) in order to illustrate the lesion location and effect on the distribution of

HRP labelled somata.

Various equations were used in the processing of the data.

$$CI = (1000 * CC) / (um * n * ILR) \quad 3.1$$

$$\%D = \left[1 - \left[(IC / 6847.8) * (36 / n) * (10 / hrp) \right] \right] \times 100 \quad 3.2$$

$$\%R = 100 * [(a - x) / a] \quad 4.1$$

Equation 4.1 rates the extent of behavioural recovery, where a is the number of ipsilateral nett rotations performed on day 7 post lesion, and x the number of ipsilateral nett rotations performed on day 32 post lesion.

Equations 3.1 and 3.2 have been described in chapter 3.

Statistical methods.

The non-parametric Mann Whitney U test was used for determining significance of changes.

In the latter series of experiments 60 min were allowed as a control period before the amphetamine injection. Analysis of the earlier data showed that of 114 30 minute control periods, 53% of the total rotations occurred in the first 20 min. To standardise data for comparative purposes the 30 min control periods were weighted by a factor of 1.859 multiplied by the

rotation score at 20 minutes. The 30 minute control period was not used per se, as it was necessary to exclude the artifactual locomotor excitation produced by the amphetamine injection, which occurred between 28 and 32 minutes.

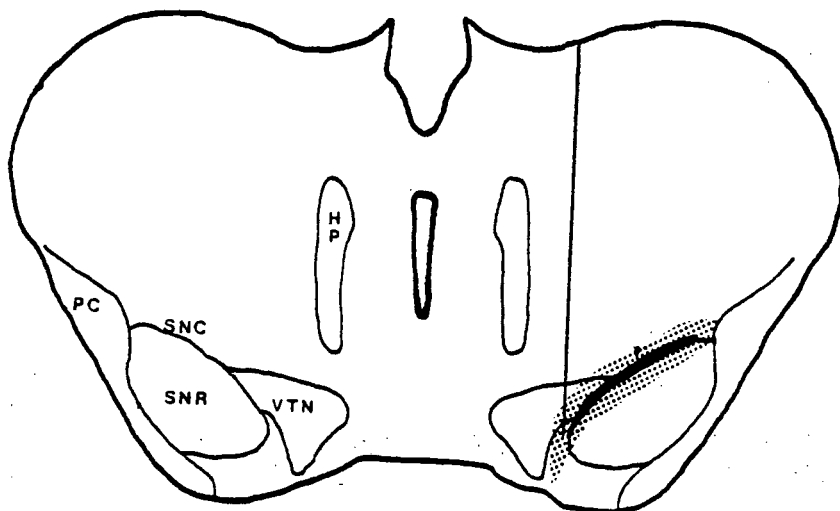
4.3 RESULTS.

4.3.1 STANDARD AND RESTRICTED 6-OHDA LESIONS OF THE SN.

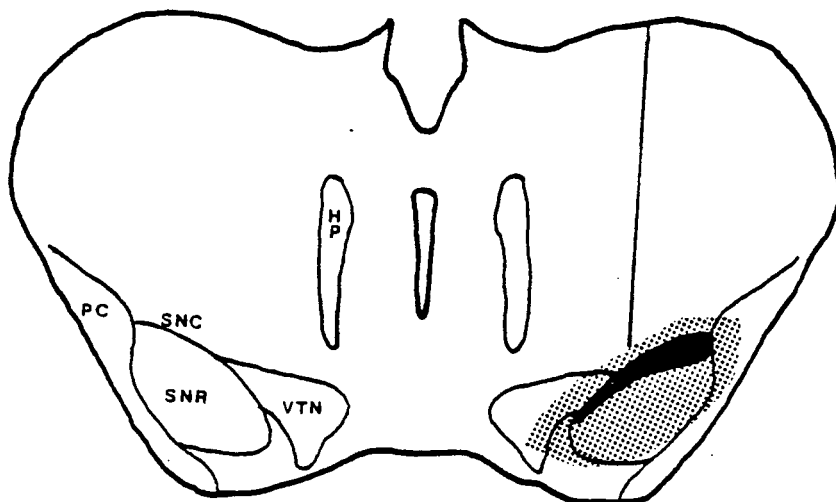
The usual lesioning protocol found in the literature is 4-5ul 6-OHDA (8-10ug) infused into a catecholaminergic target structure at a rate of 1ul/min. Fig. 4.1 illustrates the effect of a 5ul (10ug) dose as compared to a lesion of 2ul (4ug) 6-OHDA. Both lesions were effective, in that they induced rotational behaviour ipsilaterally directed with respect to the lesioned side. The 5ul injection is aimed at the central SN, AP=3.0 LAT=2.1 D=8.4. The 2ul lesion had to be moved medially to evoke ipsilaterally directed rotational behaviour. Target co-ordinates are AP=3.0 LAT=1.0 D=8.6. The 2ul lesion remains restricted to the SNC, while with the 5ul lesion, the SNR, cerebral peduncle and lateral VTA are also affected. The cellular involution seen with 2ul lesions was specifically of the compacta cell type, while the 5ul lesion produced non-specific cellular involution, and frequent vacuolation.

FIG. 4.1. Comparison of the extent of SN damage induced by 2ul (A) and 5ul (B) 6-OHDA. Diagram of mesencephalon according to Pelligrino et al. (1979), AP=3.0. The grey shaded area indicates the extent of the lesion. The vertical line represents the cannula track. The 2ul lesion is specific for the SNC, while the 5ul lesion affects other structures like the SNR, medial VTA, and PC. HP habenulo-interpeduncular tract, PC cerebral peduncle, SNC substantia nigra pars compacta, SNR substantia nigra pars reticulata, VTN ventral tegmental nucleus of Tsai.

A



B



4.3.2 THE EFFECT OF STANDARD LESIONS OF THE SN ON CIRCLING BEHAVIOUR.

5ul 6-OHDA (2ug/ul) were infused into the dominant SNC at AP=3.0 LAT=2.1 D=8.4, n=9, after two screens for spontaneous and amphetamine (1mg/Kg, i.p.) induced rotation. The dominant hemisphere lesion produced a change of rotational direction from the pre-lesion direction to that ipsilateral to the lesioned side in all subjects. Nett/total rotations (mean \pm SEM) for the two pre-lesion screens were 1.8 \pm 0.24/5.75 \pm 0.52, and 3.4 \pm 0.75/5.6 \pm 0.78 for spontaneous behaviour. 6 days post lesion the behaviour was 5.6 \pm 2.25/25.3 \pm 3.6 rotations ipsilateral to the lesioned side. 32 days post lesion there were 11.9 \pm 4.0/18.5 \pm 4.1 rotations to the ipsilateral side. The data of 6 animals are summarised in table 4.1. Mean and SEM of the data are presented in fig. 4.2. Amphetamine (1mg/Kg, i.p.) driven behaviour for the two pre-lesion screens was 10 \pm 3.3/33.3 \pm 4.7 and 18 \pm 4.6/44 \pm 7.6 rotations. 6 days post lesion there were 201.6 \pm 16.5/270 \pm 14.2 rotations ipsilateral to the lesioned side. 32 days post lesion there were 133 \pm 16.3/157 \pm 14 ipsilateral rotations. Total rotations are significantly reduced compared to day 6 post lesion (p=0.02 Mann Whitney U test). The data of 6 animals are summarised in table 4.2. Mean and SEM of the data are presented graphically in fig. 4.3.

The asymmetry index for the spontaneous screens was 12.8 \pm 9.4% and 39.2 \pm 11.3%. On day 6 post lesion AI=62.3 \pm 5.1%. 32 days post

lesion $AI=14.2\pm 12.9\%$, indicating a sharp recovery from motor asymmetry when analysed in the spontaneous condition. Due to the spread of the data this recovery is not statistically significant. The data of 6 animals are summarised in table 4.3. For amphetamine (1mg/Kg, i.p.) driven behaviour the pre-lesion screens had an AI of $26.8\pm 7.3\%$ and $39.5\pm 12\%$. On day 6 post lesion $AI=74.3\pm 4.5\%$ and 32 days post lesion $AI=80.2\pm 5\%$. The recovery from motor asymmetry suggested by the spontaneous data is thus not continued for amphetamine driven behaviour. The data of 6 animals are summarised in table 4.4.

Apomorphine induced the classical contralateral rotation syndrome. Apomorphine (1mg/Kg, i.p.) induced rotation was measured on day 8 post lesion. $130\pm 31.3/184\pm 26.3$ contralateral rotations were recorded. Some animals responded poorly to apomorphine (AS43, AS55, AS57). This can be attributed to damage to the descending striatonigral fibres in the case of AS43. AS43 had a large lesion affecting the cerebral peduncle, which contains the striatonigral projection (see fig. 4.4). Interruption of the striatonigral projection ipsilateral to the lesion would result in rotation to that side with apomorphine rather than contralaterally. AS55 and AS57 had their lesions rather anterior and lateral (fig. 4.4). Their lesions can thus only be described as partial SN lesions, in spite of the large dose of neurotoxin administered. There would thus not have been sufficient DA depletion of the ipsilateral striatum to induce the striatal supersensitivity responsible for contralateral circling behaviour with apomorphine. This result underscores the importance of lesion location and size in inducing

robust ipsilateral rotational behaviour without affecting striatal output projections.

A schematic description of the lesion sites is included in fig. 4.4.

The next 5 sections examine the role of discrete sites within the ventral mesencephalon in the generation of rotational behaviour ipsilateral to the lesioned side. The areas individually tested with restricted lesions were all globally affected by the standard lesion. The lateral SNC was suggested as potentially important as this area was often affected by the standard 6-OHDA lesion as determined by histological analysis. The effect of its discrete lesion with 6-OHDA is examined in section 4.3.3. The DTV was considered an interesting lesion site as this is the site of crossed projection decussation. The crossed projection is known to be catecholaminergic, and a 6-OHDA lesion had been seen to disrupt it (chapter 3). The behavioural consequences of this are examined in section 4.3.4. Standard 6-OHDA lesions often induce involution and gliosis of the ventral SN. In section 4.3.5 the behavioural effect of a discrete ventral SN lesion without SNC involvement is reported. Standard 6-OHDA lesions often affect the cluster of cells just medial to the SNC, within the VTN. The effect of discrete 6-OHDA lesioning of this area is reported in section 4.3.6. In addition, the crossed projection can be damaged by standard lesions (Douglas et al. 1984). The main aim of the various restricted lesions of the ventral mesencephalon was to find a lesion site which induced ipsilateral rotation while

sparing the crossed projection. In this way the role of the crossed projection in recovery can be evaluated.

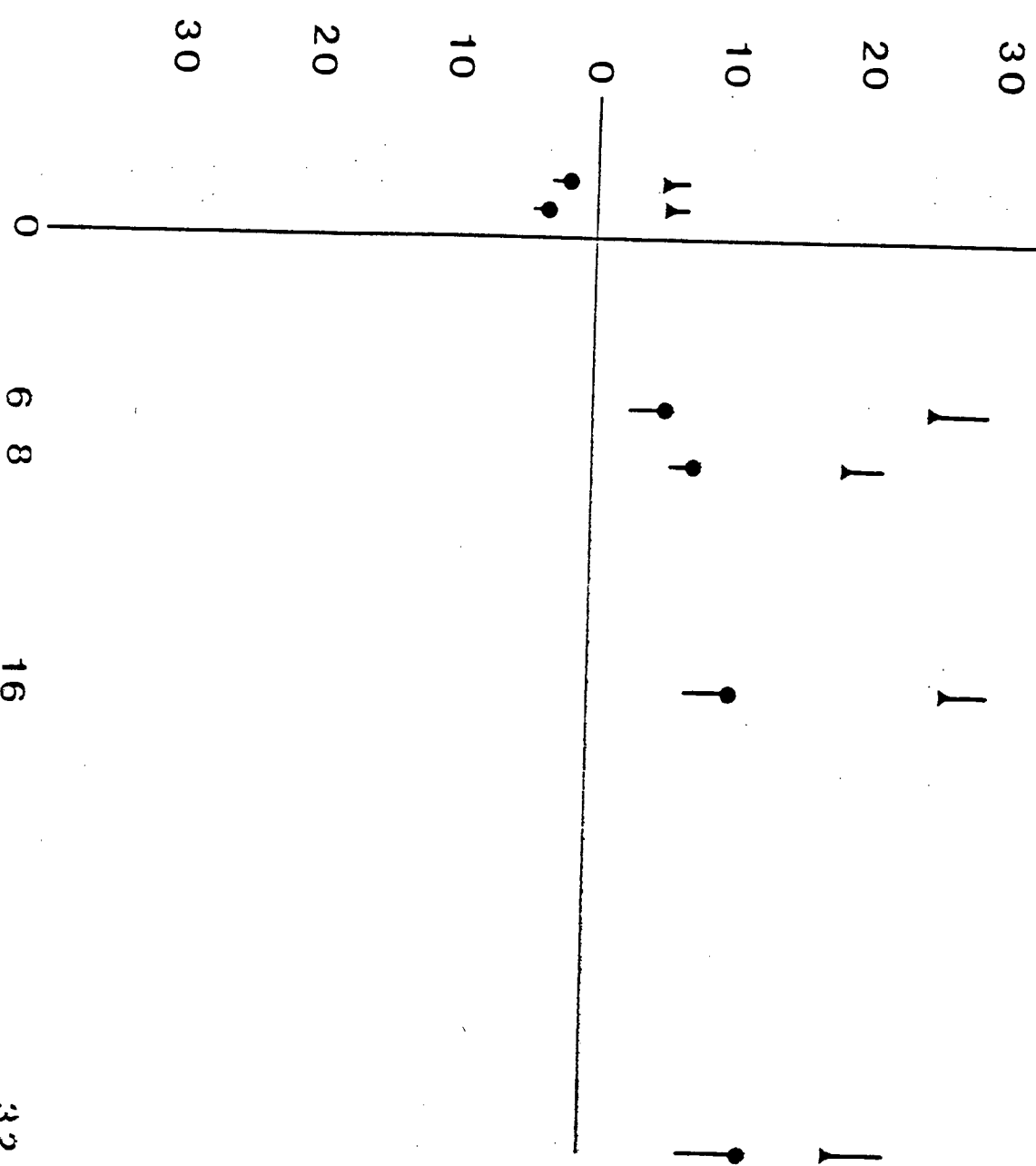
Table 4.1 The effect of a standard 6-OHDA lesion of the SN on spontaneous rotational behaviour. Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 6, 8, 16 and 32 are the days on which behaviour was tested post-lesion. n=6. The mean and SEM of this data are presented graphically in fig 4.2. * Lesioned in the non-dominant hemisphere.

RAT	AP	LAT	DEPTH	S1	S2	6	8	16	32
AS32	3.6	2.0	8.5	-4/9	-1.5/3	12/14	24/26	21.5/29.5	-2.5/9
AS43	3.4	2.1	8.6	-1.5/5.5	-3.5/3.5	-19.5/37	-2.5/44.5	-2.5/46.5	11/15
AS46	3.6	2.4	8.4	-2/6	-10.5/14	515/15	8/8	8/11	59/68.5
AS53	3.6	2.1	8.5	-1.5/9	-6.5/7.5	4/5	2/15	3/9	-4/4
AS55	3.6	1.9	8.7	-2.5/4.5	0/3	4.5/16.5	10/11	35/39.5	9/11
* AS57	4.2	2.1	8.6	0.5/0.5	2/2.5	18/64.5	5.5/8.5	-10/24	-1/3.5
Nett	3.7	2.1	8.6	-1.8	-3.4	5.6	7.8	10.2	11.9
+--SEM	0.05	0.03	0.02	0.24	0.75	2.25	1.5	3.0	4.0
Total				12.8	39.2	62.3	59.3	41.2	14.2
+--SEM				9.4	11.3	5.1	7.5	9.4	12.9

Nett or total rotations on days 6, 8, 16 or 32 post-lesion not significantly different from S1 $p=0.155$ Mann Whitney U test. Total rotations on day 6 not significantly different from 32.

FIG. 4.2. Schematic representation of the spontaneous rotational responses to standard 6-OHDA lesions. Fig. of data presented in table 4.1. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 6, 8, 16, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=6$. The lesion results in crossing of rotational direction from the pre-lesion direction to ipsilateral to the lesioned side. There is no diminution of the response over the test period.

TOTAL ROTATIONS
CONTRA NETT IPSI



DAYS

Table 4.2 The effect of a standard 6-OHDA lesion of the SN on amphetamine (1mg/Kg, i.p.) driven rotations. The effect of apomorphine (1mg/Kg, i.p., day 8 only) is presented. Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 6, 8, 16 and 32 are the days on which behaviour was tested post-lesion. n=6. The mean and SEM of this data are presented graphically in fig 4.3.
** Lesioned in the non-dominant hemisphere.*

RAT	AP	LAT	DEPTH	S1	S2	6	8	16	32
AS32	3.6	2.0	8.5	-6/25	-68/77	172/360	-446/454	336/336	203/207
AS43	3.4	2.1	8.6	-4/8	-10/11	220/232	47/76	141/190	26/42
AS46	3.6	2.4	8.4	-43/88	-5/11/25	365/379	-149/208	106/111	272/279
AS53	3.6	2.1	8.5	-6.5/18	-28/30.5	158/171	-234/243	50/52	88/90
AS55	3.6	1.9	8.7	-17.5/30	-6.25/121	229/280	3/4	145/151	160/162
*AS57	4.2	2.1	8.6	17/30	11/12	65/199	-1/8	33/55	42/160
Nett	3.7	2.1	8.6	-10	-18	201.6	-130	135	133
+--SEM	0.05	0.03	0.02	3.3	4.6	16.5	31.5	18.1	16.3
Total				33.3	44	270	184	149	157
+--SEM				4.7	7.6	14.2	26.3	17.7	14

Nett rotations on day 32 post-lesion significantly different from S1, $p=0.001$.

Total rotations on day 32 significantly different from S1, $p=0.002$ Mann Whitney U test.

Total rotations on day 6 post-lesion significantly different from day 32, $p=0.02$ Mann Whitney U test.

FIG. 4.3. Schematic representation of the rotational responses of animals with standard lesions to amphetamine (1mg/Kg, i.p.), and apomorphine (1mg/Kg, i.p., day 8 only). Fig. of data presented in table 4.2. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 6, 8, 16, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=6$. The lesion results in crossing of rotational direction from the pre-lesion direction to ipsilateral to the lesioned side. There is a trend towards diminution of the total rotation response over the test period ($p=0.02$). The apomorphine response at day 8 is contralateral to the lesioned side.

TOTAL ROTATIONS
CONTRA NETT IPSI

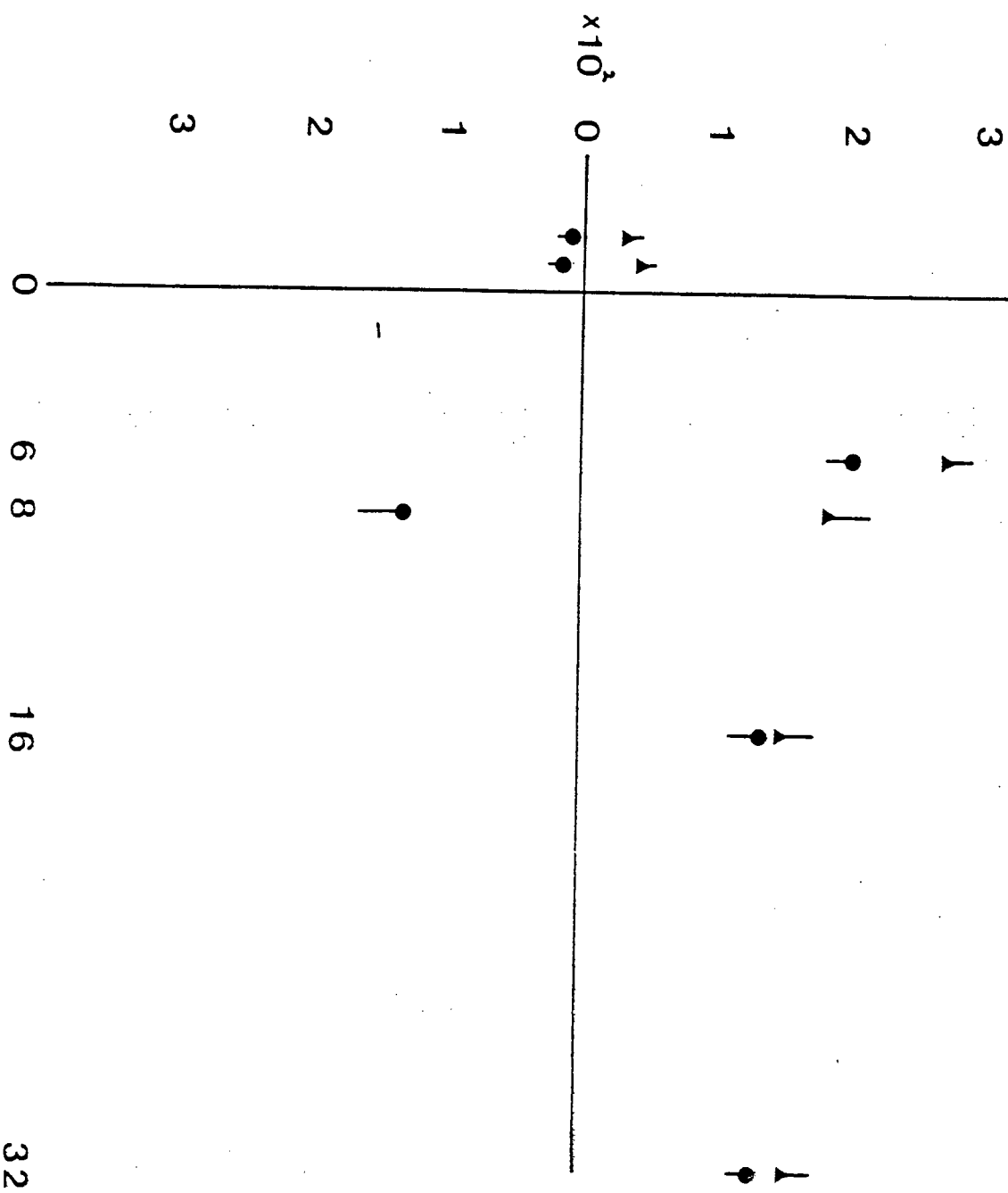


Table 4.3 Table of the asymmetry index (Nett/Total*100) of spontaneous rotations.

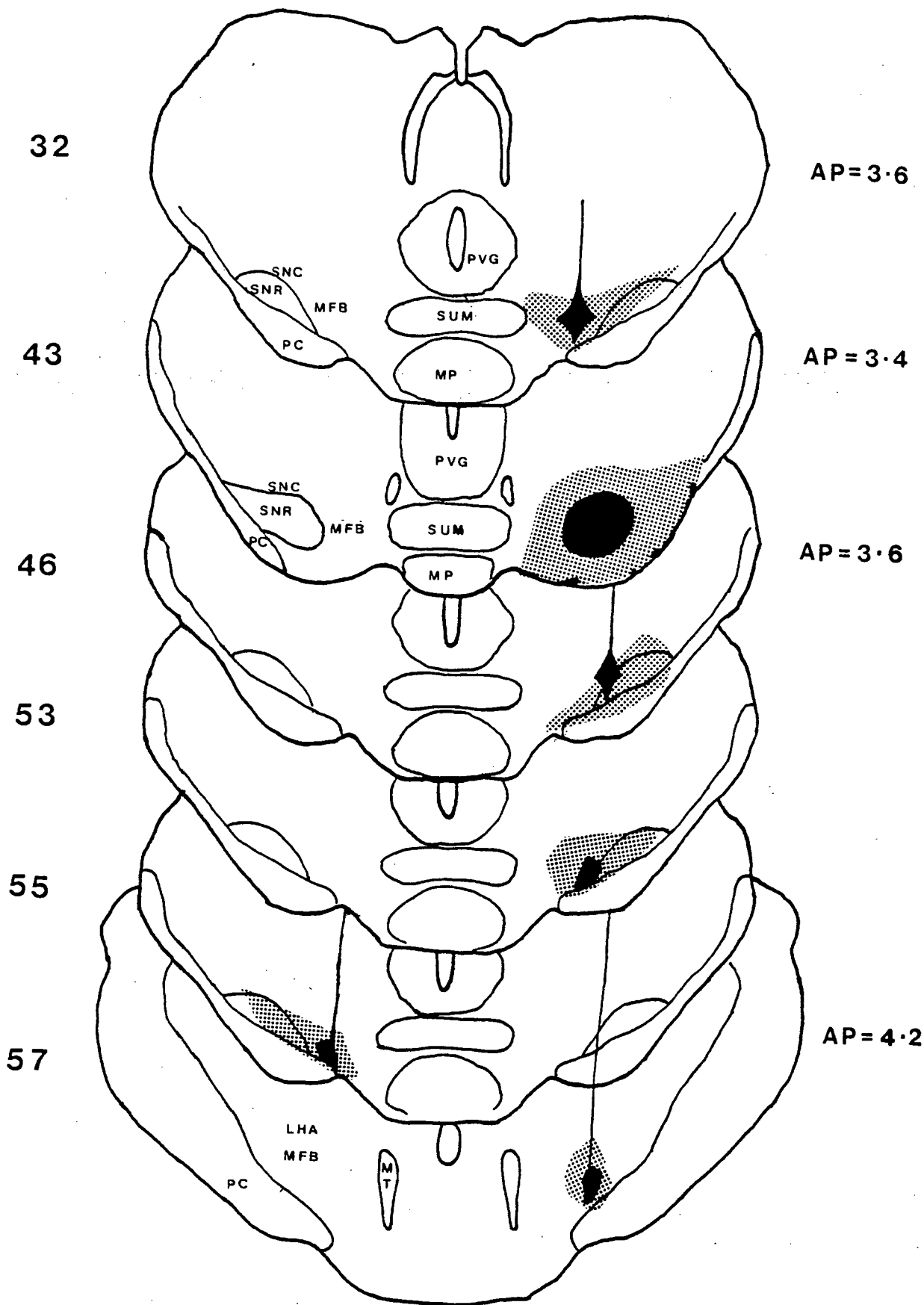
S represents the screens, while 6, 8, 16 and 32 are the days on which behaviour was tested post-lesion. n=6.

RAT	S1	S2	6	8	16	32
AS32	-44.4	-60	85.7	92.3	100	-27.5
AS43	-27.2	-100	-52.7	-5.6	-5.4	73.3
AS46	-33.3	-69	100	100	72.7	86.1
AS53	-16.6	-86.6	80	13.3	33.3	-100
AS55	-55.5	0	27.3	90.9	88.6	81.8
AS57	100	80	279	64.7	-41.6	-28.6
Mean	-12.8	-39.2	44.7	59.3	41.2	14.2
+--SEM	9.4	11.3	9.4	7.5	9.4	12.9

Table 4.4 Table of asymmetry index (Nett/Total*100) for amphetamine (1mg/kg, i.p) and apomorphine (1mg/Kg, i.p., day 8 only) driven rotations. S represents the screens, while 6, 8, 16 and 32 are the days on which behaviour was tested post-lesion. n=6.

RAT	S1	S2	6	8	16	32
AS32	-24.7	-88.3	47.7	-98.2	100	98.1
AS43	-51.5	-91.5	94.8	62.5	74.2	62.6
AS46	-48.9	-46.6	96.3	-71.6	95.4	97.5
AS53	-36.1	-93.4	92.4	-96.2	96.1	97.8
AS55	-58.3	-5.2	81.8	76.4	96	98.8
AS57	58.7	88.2	33	-12.5	60	26.25
Mean	-26.8	-39.5	74.3	-23.3	87	80.2
+--SEM	7.3	12	4.5	13	2.9	5

FIG. 4.4. Locations of standard 6-OHDA lesions of the SN, n=6. Sections conform to Pelligrino et al. (1979), AP=3.4, 3.6, and 4.2. Black areas show regions of non-specific damage in the form of severe gliosis and vacuolation, grey areas represent regions of cellular involution. The lesions are generally widespread, including structures other than the SN, notably the MFB, SNR and PC. There is also a marked degree of non-specific damage in the form of vacuolation. LHA lateral hypothalamus, MFB medial forebrain bundle, MP posterior mamillary nucleus, MT mamillothalamic tract, PC cerebral peduncle, PVG periventricular grey, SNC substantia nigra pars compacta, SNR substantia nigra pars reticulata, SUM supramamillary nucleus.



4.3.3 RESTRICTED 6-OHDA LESIONS OF THE LATERAL ASPECT OF SN.

2ul (4ug) 6-OHDA were infused into the dominant lateral SNC after two screens for spontaneous and amphetamine (1mg/Kg, i.p.) induced rotation. Of 12 subjects, 4 (97, 109, 139, 143) showed crossing of rotational direction post lesion for the assessment of spontaneous behaviour (table 4.5). Only 3 (92, 97, 139) responded in this way for amphetamine-driven behaviour (table 4.6). For spontaneous rotation, 97 showed recovery to its pre-lesion direction of rotation, from 20 turns (ipsilateral to the lesion) on day 7, to 2 turns (contralateral to the lesion) on day 32. For amphetamine induced rotation, all three subjects that showed crossing also showed recovery from nett ipsilateral rotations. Number 92 recovered from 70 *contralaterally* directed rotations to 3 *ipsilaterally* directed, ie. in the pre-lesion direction. 97 and 139 did not show such profound recovery, but exhibited the same trend (table 4.6).

The remainder of the subjects showed no crossing of rotational direction, though an increase in the number of total rotations (14 ± 0.7) on day 1 to 22 ± 1.75 (mean \pm SEM) on day 24 post lesion for spontaneous behaviour, and 37 ± 3.5 on day 1 to 112 ± 5.1 on day 24 post lesion for amphetamine-driven behaviour in response to the lesion. The increases for spontaneous behaviour were not statistically significant ($p > 0.1$), but those for amphetamine induced rotation were significant ($p < 0.002$, Mann Whitney U tests). The data of 12 animals is presented in tables 4.5 and 4.6. Mean

and SEM of the data are represented in fig. 4.5 and 4.6. Table 4.7 and 4.8 present the AI of the spontaneous and amphetamine-induced rotation of animals with lateral SNC lesions. AI for spontaneous rotation was unchanged over the test period (table 4.7). AI for amphetamine induced rotation was increased on day 24 post lesion compared to the first screen ($p < 0.02$), but not the second screen (table 4.8). Thus, the lateral SNC lesion had a minimal effect on asymmetry, only resulting in hyperactivity to amphetamine. Lesion locations of each individual animal are represented in fig. 4.7. The mean \pm SEM lesion location was AP=2.6 \pm 0.03 LAT=2.8 \pm 0.05 D=8.9 \pm 0.06. The lesions were restricted to the lateral third of the SNC, and did not affect any other structures. The lesion was specific for the large compacta type cells, and there was little non-specific damage in the form of vacuolation or gliosis.

One non-crossing animal (1401) was injected with HRP in the striatum ipsilateral to the lesion and the cell count of the lesioned SN revealed labelling similar to controls. Labelled cells were seen in the contralateral DTV, VTA and SN. Cell counts are described in table 4.9, and illustrated by camera lucida drawings in fig. 4.8.

From the behavioural results and histological descriptions it can be concluded that 6-OHDA lesions of the lateral SNC are relatively ineffective in inducing rotational behaviour ipsilateral to the lesion. They do, however, induce hyperactivity, which manifests itself as increased total and nett contralateral rotations. There is no recovery from the lesion-induced hyperactivity.

Table 4.5. The effect of restricted 6-OHDA lesioning of the anterolateral SN on spontaneous rotational behaviour. Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14, 24 and 32 are days on which behaviour was tested post-lesion. n=12. The mean and SEM of this data are graphically represented in fig 4.5. * Lesioned in non-dominant hemisphere.

RAT	AP	LAT	D	S1	S2	7	14	24	32
*92	3.0	2.8	8.1	12/16	7/20		33/42	-6/26	11/39
96	3.0	3.1	8.4	-15/22	-18/24		-24/24	-61/71	-8/20
97	3.0	2.8	7.6	-2/18	-4/35		20/33	20/51	-4/20
99	3.0	3.5	8.0	-7/11	-4/13		-13/19	-13/20	-13/13
104	2.4	3.2	8.9	-0/4	-0.5/2.5	-3.5/16.5	-1.5/11	-0.5/1.5	
106	2.4	3.2	9.2	-15/26	-2/8	-1/3.5	-1.5/4.5	-1.5/1.5	
(*)107	2.4	2.8	9.1	3.5/13	1/5	5.5/14.5	9/16.5	1/25.5	
109	2.4	3.0	9.0	-5.5/25	-11/31.5	-18/22	-14.5/22	2/9	
110	2.4	3.1	9.6	-0/0	-4/6	-2/3.5	-3/11	-0/8	
112	2.4	3.0	9.6	-1/4.5	-3.5/3.5	-7.5/18	-14.5/26	-2/9	
139	2.4	1.6	9.6	-6/18	-7.5/8.5	13/26	11/24	20/31.5	
143	2.4	1.5	9.6	9/16	6/13.5	0.5/30	-14.5/119	-1/10	
Nett	2.6	2.8	8.9	-1.3	-3.4	-1.6	-1	-4.2	-3.25
+SEM	0.03	0.05	0.06	0.7	0.6	0.8	1.4	1.9	0.9
Total				14	13.4	16.8	29	22	22.8
				0.7	1.0	0.8	2.5	1.75	0.9

Rotational behaviour post-lesion not statistically different from pre-lesion results $p > 0.1$ Mann Whitney U tests.

FIG. 4.5. Schematic representation of the spontaneous rotational responses to restricted anterolateral SNC lesions. Fig. of data presented in table 4.5. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=12$. There is an increase in total rotations which is not significant, but no change of rotation direction from contralateral to ipsilateral to the lesioned side.

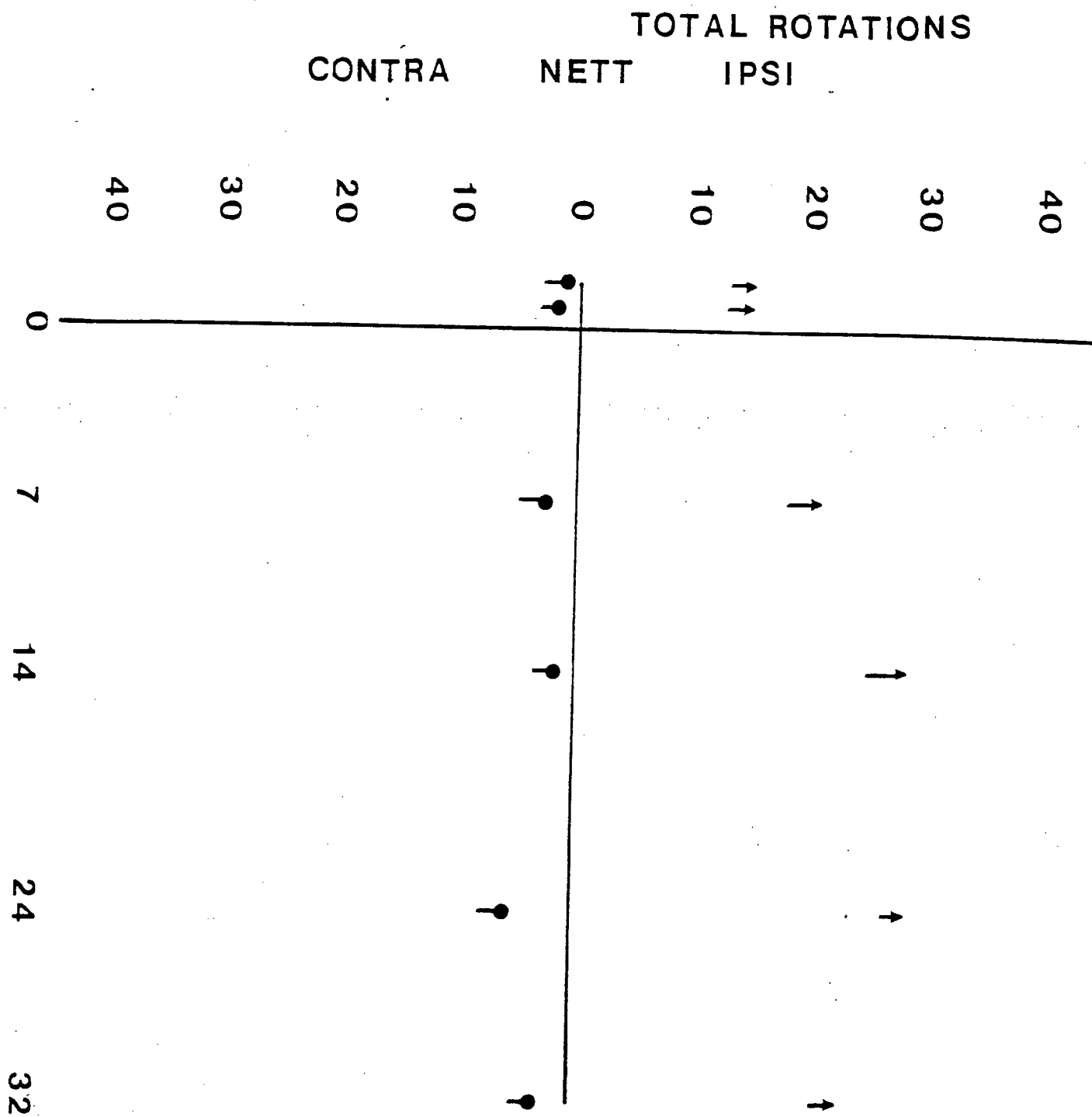


Table 4.6 The effect of restricted 6-OHDA lesion of the anterolateral SN on amphetamine (1mg/kg, i.p.) driven rotational behaviour. Data presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14, 24 and 32 are the days on which behaviour was graphically represented in fig 4.6.

RAT	AP	LAT	D	S1	S2	7	14	24	32
92	3.0	2.8	8.1	6/29	2/53		-70/135	-47/68	3/45
96	3.0	3.1	8.4	-30/40	-59/59		-76/77	-100/101	-14/26
97	3.0	2.6	7.6	-2/21	-56/65		104/123	83/90	110/125
99	3.0	3.5	8.0	-7/15	-15/20		-76/77	-49/51	-146/147
104	2.4	3.2	8.9	1/17	-12/19	-37/42	-52/54	-89/106	
106	2.4	3.2	9.2	-5/17	-0.5/2	-122/127	-124/124	-109/112	
107	2.4	3.1	9.1	-2/5	-0.25/7	0.75/14	-62/64	-10/27	
109	2.4	3.0	9.0	-135/164	-286/300	-212/225	-12/18	-182/194	
110	2.4	3.1	9.6	-20/26	-11/23	-45/55	-65/67	-62/63	
112	2.4	3.0	9.6	-14/29	-70/70	-21/38	-96/98	-163/172	
139	2.4	1.6	9.6	-24/58	-132/142	268/273	254/263	230/234	
143	2.4	1.5	9.6	6/30	-85/90	-88/173	-133/407	-38/129	
Nett	2.6	2.8	8.9	-18	-60	-32	-34	-44.6	11.75
+SEM	0.03	0.05	0.06	3.2	6.8	11.5	9.5	9.2	8.6
Total				37.5	70.8	118	116	112	85
+SEM				3.5	6.8	8.1	9.3	5.1	4.9

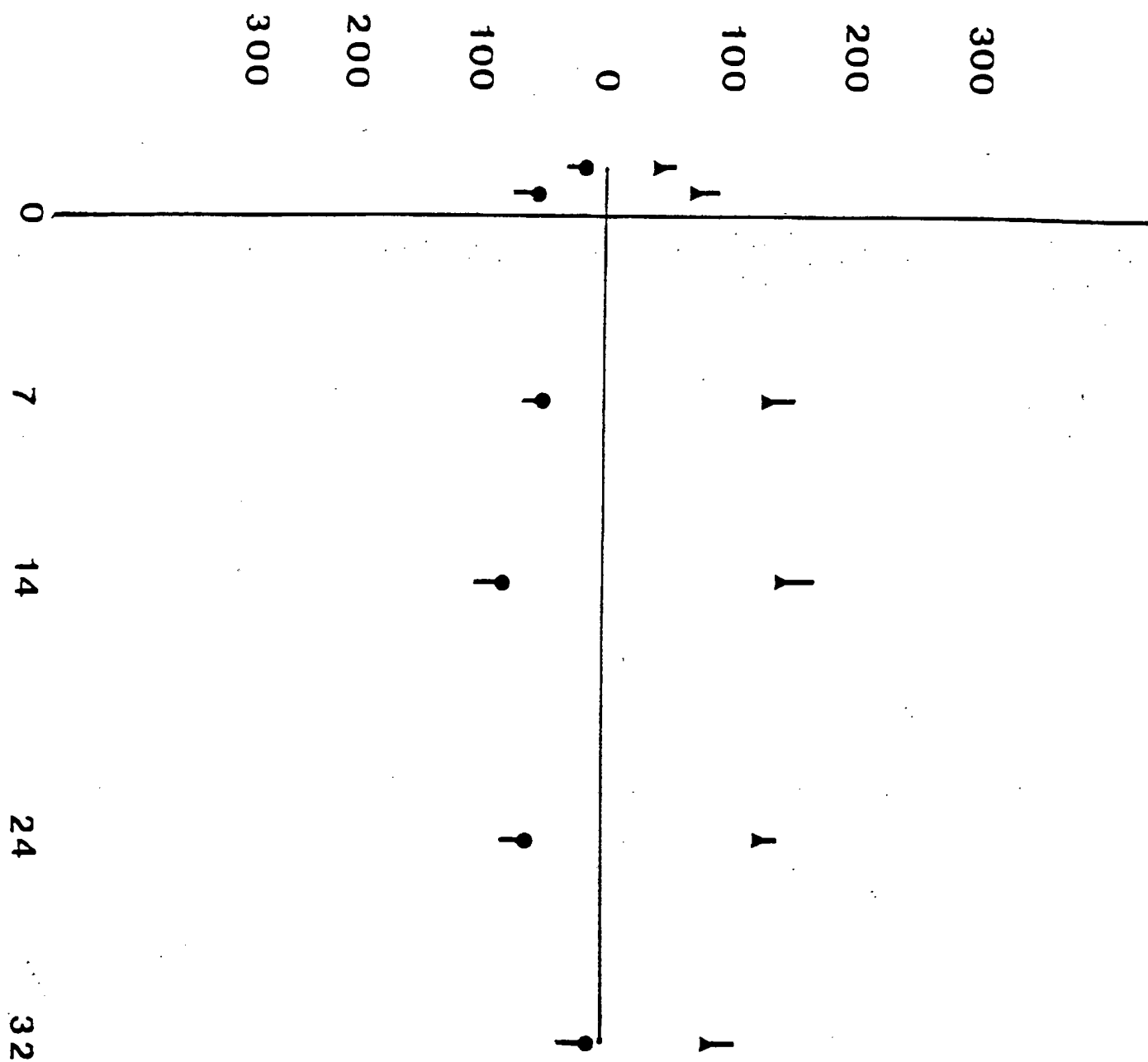
Nett rotations on day 24 significantly different from S1 $p < 0.05$.

Total rotations on day 24 significantly different from S1 $p < 0.002$.

Mann Whitney U tests.

FIG. 4.6. Schematic representation of the rotational responses of animals with restricted anterolateral SNC lesions to amphetamine (1mg/Kg, i.p.). Fig. of data presented in table 4.6. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles show total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=12$. There is no change of rotation direction from contralateral to ipsilateral to the lesioned side, though an increase in the number of contralateral rotations is apparent ($p<0.05$). Total rotations are increased by the lesion ($p<0.002$).

TOTAL ROTATIONS
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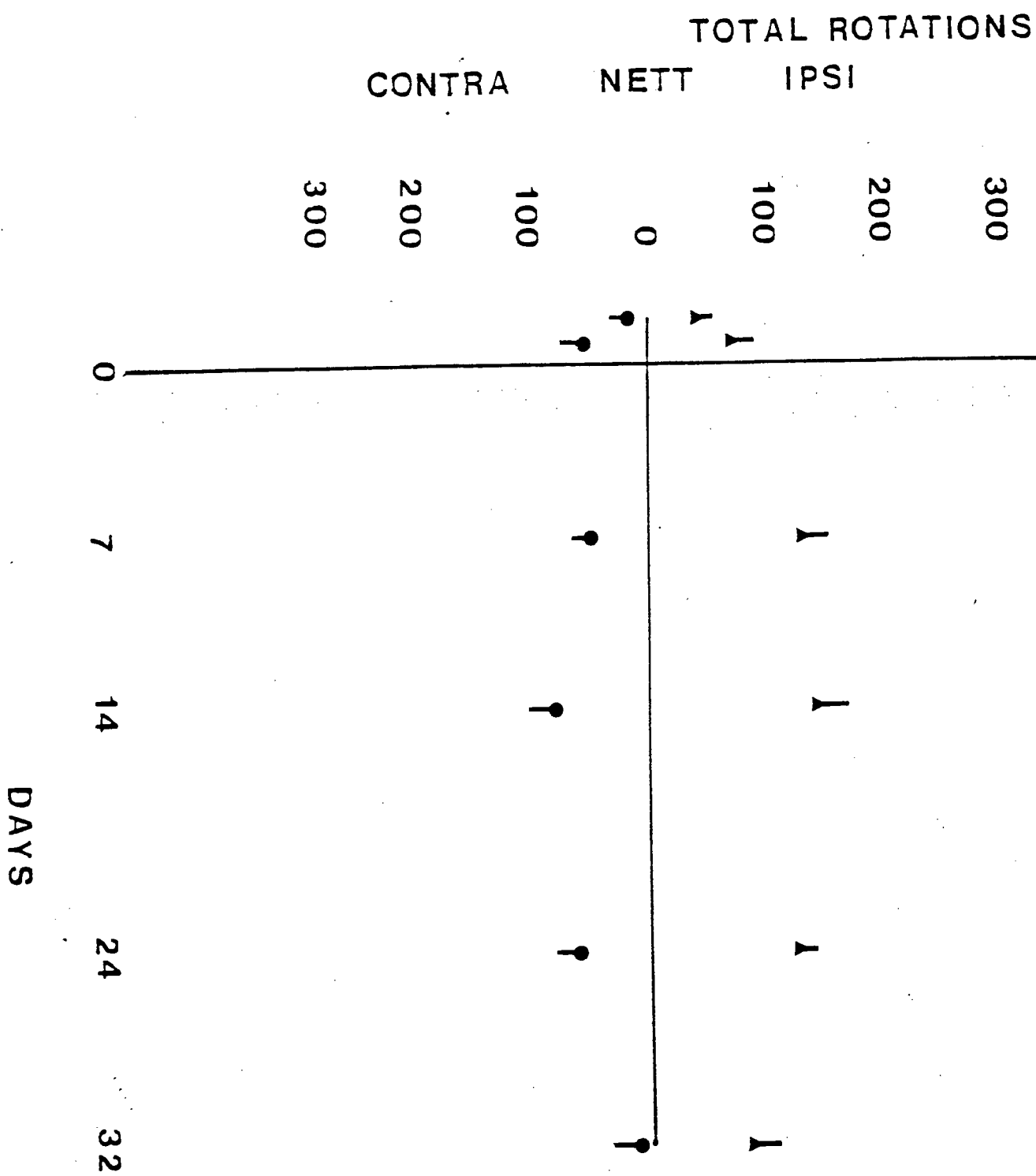


Table 4.7 Table of the asymmetry index (Nett/Total*100) of spontaneous rotations with anterolateral SNC lesion. S represents the screens, while 6, 8, 16 and 32 are the days on which behaviour was tested post-lesion. n=12.

RAT	S1	S2	7	14	24	32
92	39	38		76	-19	29
96	-67	-77		-100	-97	-33
97	-11	-8		-62	-38	21
99	-74	-23		-65	-60	-100
104	0	20	-22	-13	-33	
106	-58	-25	-25	-40	-100	
107	38	9	37	54	4	
109	-22	-35	-80	-67	26	
110	0	69	-50	-2.5	0	
112	-20	-100	-40	-57	-20	
139	-33	-89	53	49	65	
143	6	45	2	-12	-9	
Mean	-16.8	-14.6	-15.6	-20	-23.4	-20.8
+SEM	3	4.5	5.6	4.6	3.9	14.9

No significant differences.

Table 4.8 Table of asymmetry index (Nett/Total*100) of amphetamine (1mg/Kg, i.p.) driven rotations with lateral SNC lesion. S represents the screens, while 7, 14, 24 and 32 are the days on which behaviour was tested post-lesion. n=12.

RAT	S1	S2	7	14	24	32
92	21	2		-52	-69	7
96	-75	-100		-99	-99	-53
97	-26	-85		-84	-92	-88
99	-44	-75		-99	-97	-99
104	4	-65	-88	-95	-84	
106	-28	-25	-96	-100	-97	
107	-44	-3	5	-97	-38	
109	-83	-95	-94	-66	-94	
110	-75	-49	-99	-98	-99	
112	-49	-100	-56	-98	-95	
139	-42	-93	98	97	98	
143	21	-94	-51	-33	-29	
Mean	-35	-65.2	-47.6	-68.6	-68.6	-58.3
+SEM	2.9	3.1	8.5	4.7	4.7	11.9

AI on day 24 significantly different from S1. $p < 0.02$ Mann Whitney U test.

FIG. 4.7. Locations of restricted 6-OHDA lesions of the lateral SN. n=12. Sections conform to Pelligrino et al. (1979, AP=3.0 and 2.4). The lesions were restricted to the lateral third of the SN, and did not affect any other structures. Black indicates non-specific damage in the form of severely coagulated or vacuolated tissue, while grey indicates the extent of the lesion effect. Involuting cells were seen within the grey area, sometimes alongside healthy cells. There was little vacuolation and the lesion was specific for the large compacta type cells. DTV ventral tegmental decussation, HP habenulo-interpeduncular tract, IP interpeduncular nucleus, LM medial lemniscus, SN substantia nigra, VTN ventral tegmental nucleus of Tsai

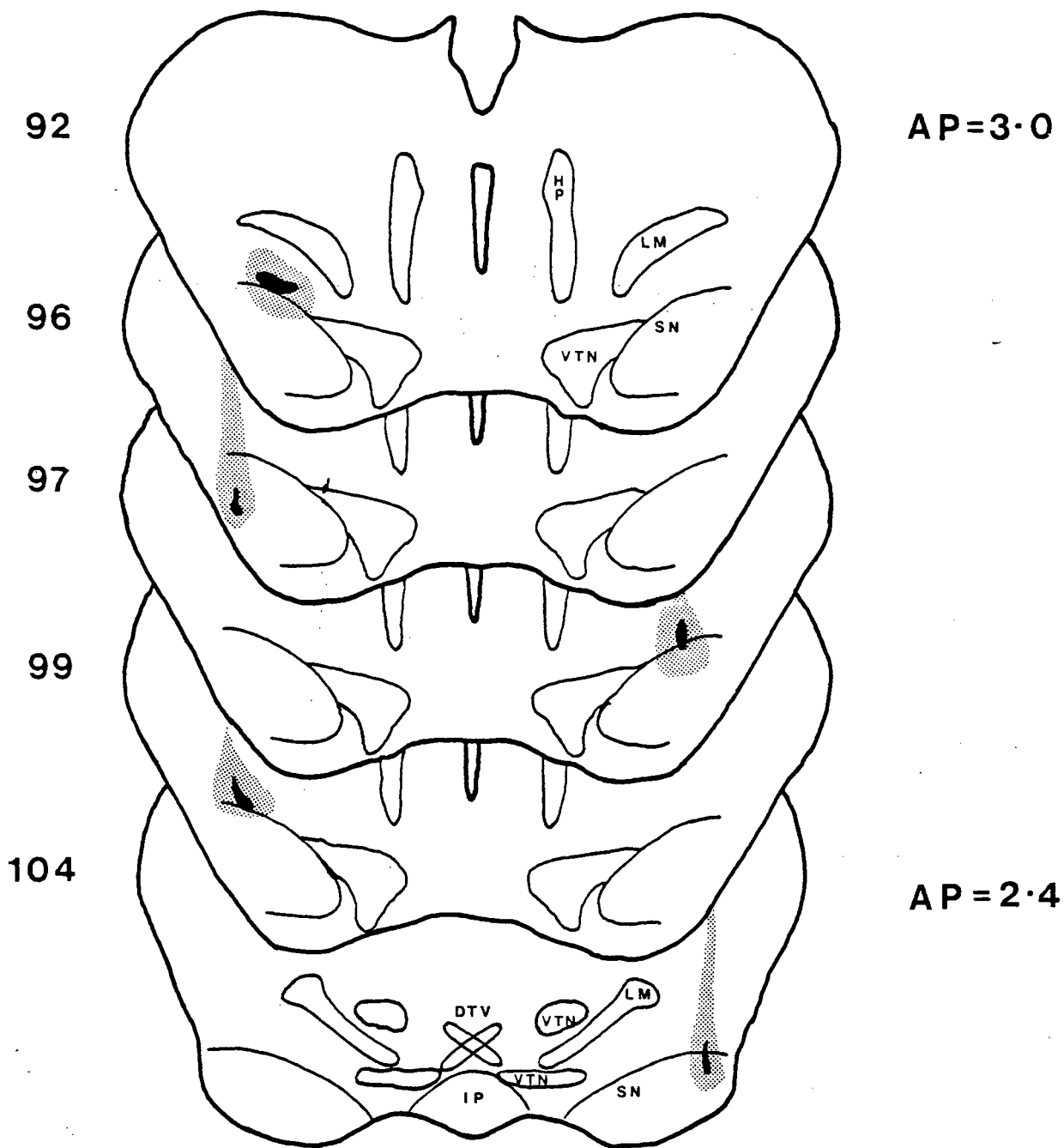


Fig. 4.7 continued.

106

AP=2.4

107

109

110

112

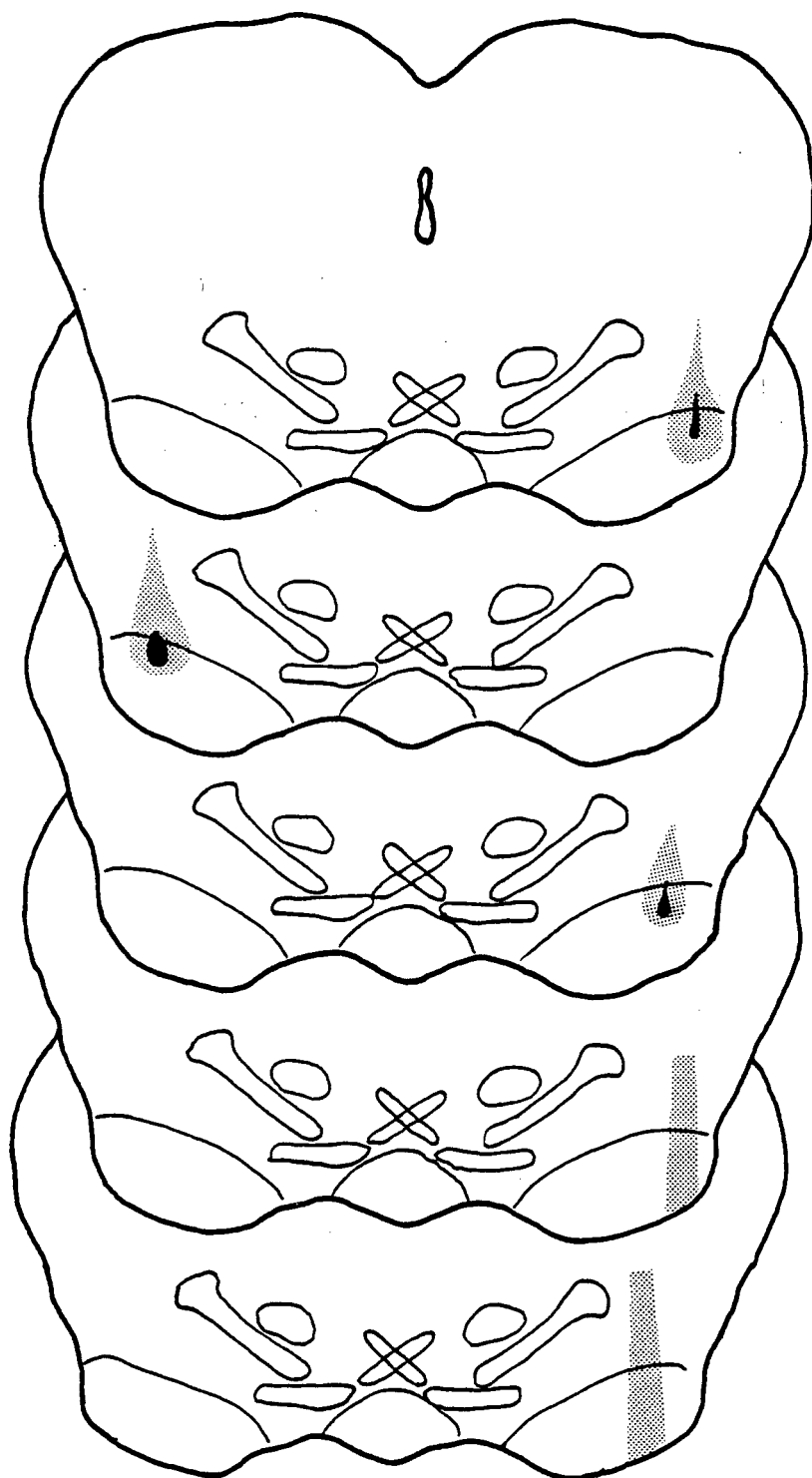


Fig. 4.7 continued.

139

AP = 2.4

143

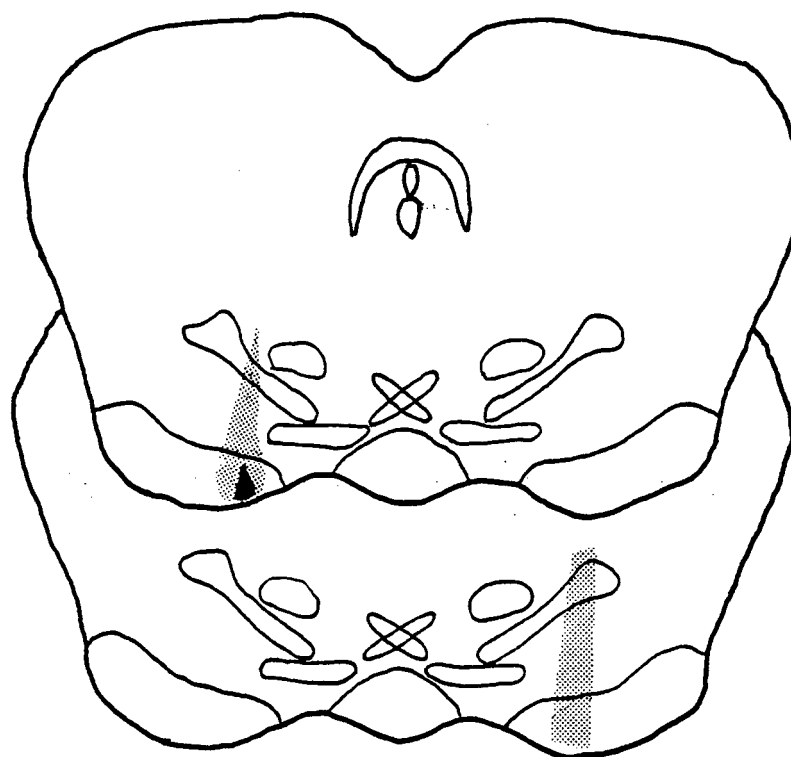


Table 4.9 RAT 110 Ventral mesencephalic HRP labelled cell counts of rat 110. Ventral mesencephalon retrogradely labelled from a striatal deposition site. Restricted 6-OHDA lesion of the lateral SNC AP=2.4 LAT=3.2 D=9.2. HRP labelled cells distributed similarly to controls, see chapter 3. Camera ludida diagramatic presentation of this data is in fig 4.8.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	0	0	0	0	0	2
3.4	1	0	0	0	0	0	85
3.0	1	0	0	0	8	90	135
2.6	2	0	8	5	9	46	154
2.2	1	2	6	3	31	52	76
1.8	1	1	4	2	26	41	94
TOTALS	7	3	18	10	74	229	546

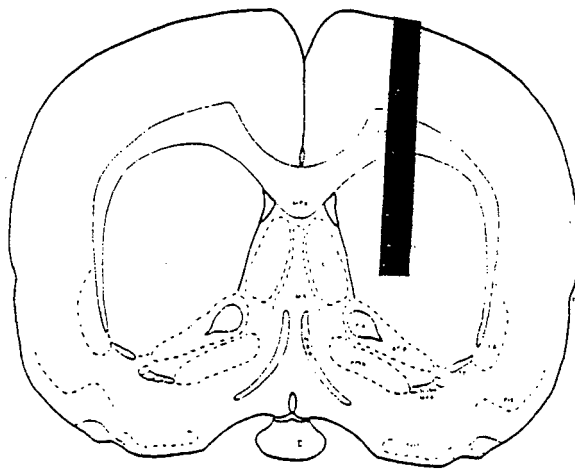
Total ipsilateral cells 849

Total contralateral cells 31

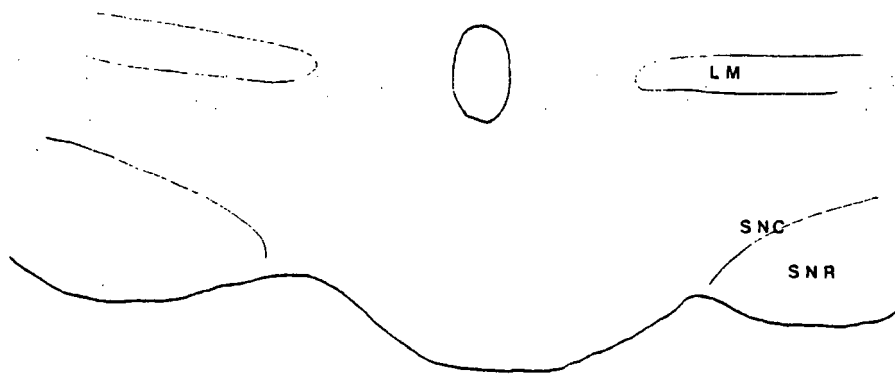
CL/IL*100=3.7%

CI=8.6

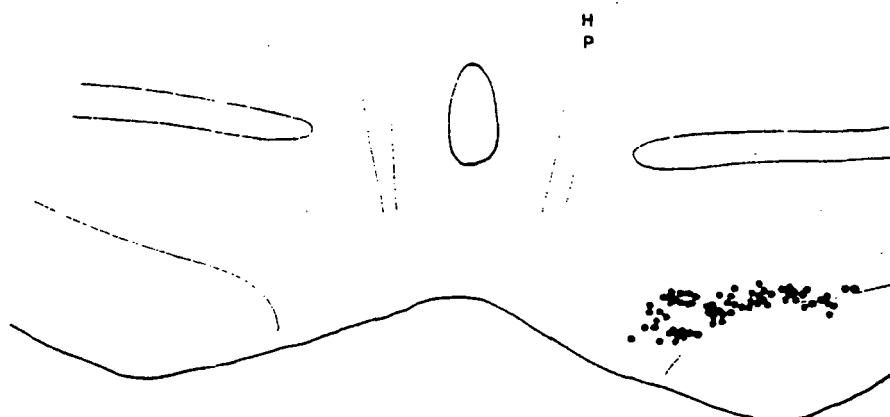
FIG 4.8 Schematic representation of the typical distribution of HRP filled cells in rats with restricted lateral SN lesions. This schematic is of the data of rat 110 tabulated in table 4.7. The diagrams are camera lucida drawings of the histological section closest to the relevant Pelligrino et al. (1979) section, AP indicated at top right. HRP labelled cells are represented by closed circles. The striatal HRP deposition site is shown above. The ventral mesencephalic distribution of HRP labelled cells is indistinguishable from controls, indicating that the lateral lesion had a negligible effect on the pattern of labelling. The lesion is the stippled region in the lateral SN AP=2.6. DTV ventral tegmental decussation, HP habenulo-interpeduncular tract, IP interpeduncular nucleus, LM medial lemniscus, SN substantia nigra



3.6



3.4



3.0

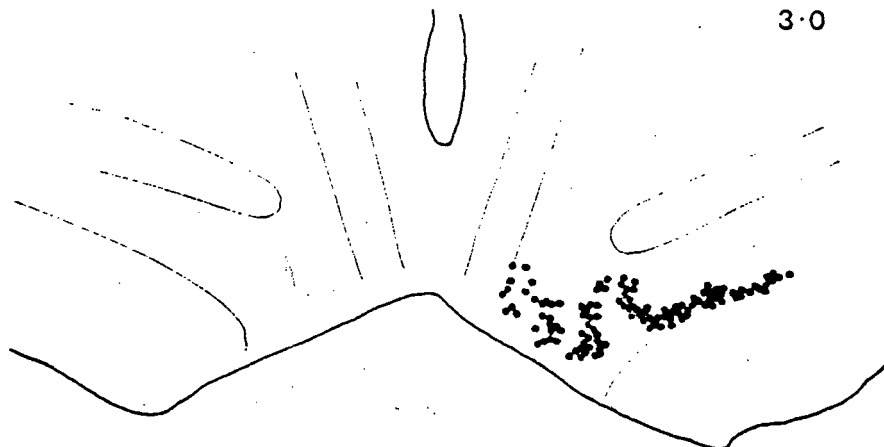
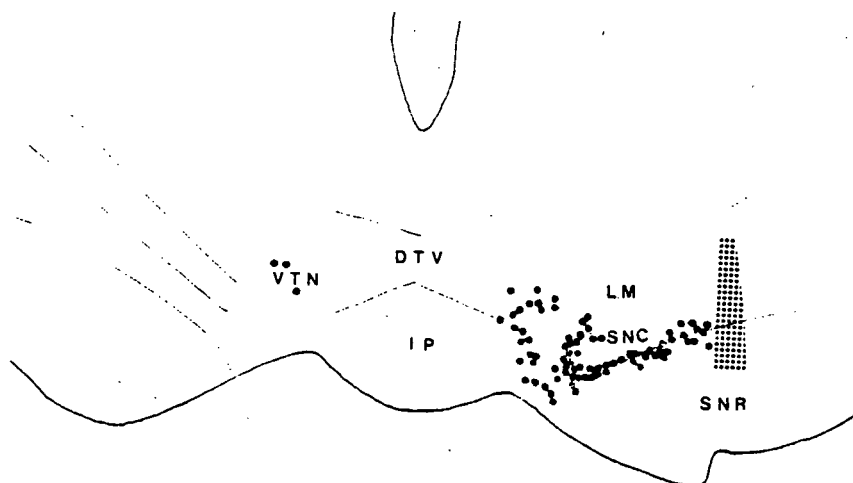
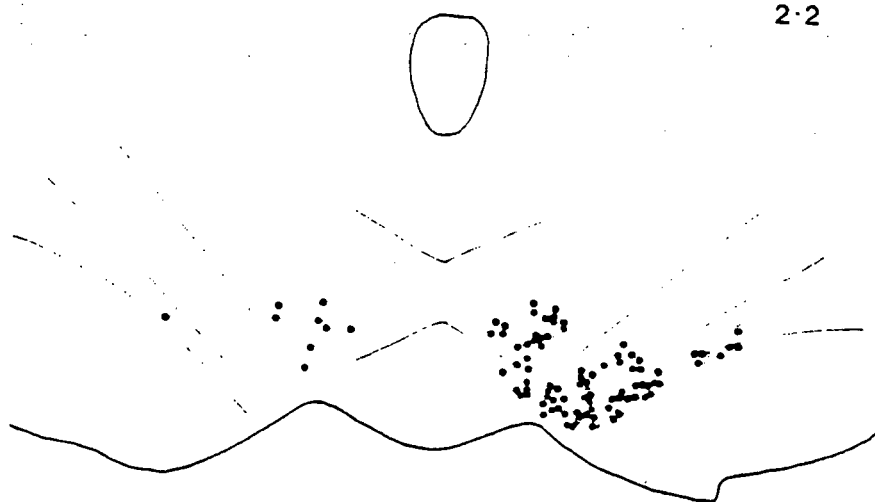


Fig. 4.8 continued.

2.6



2.2



2.0

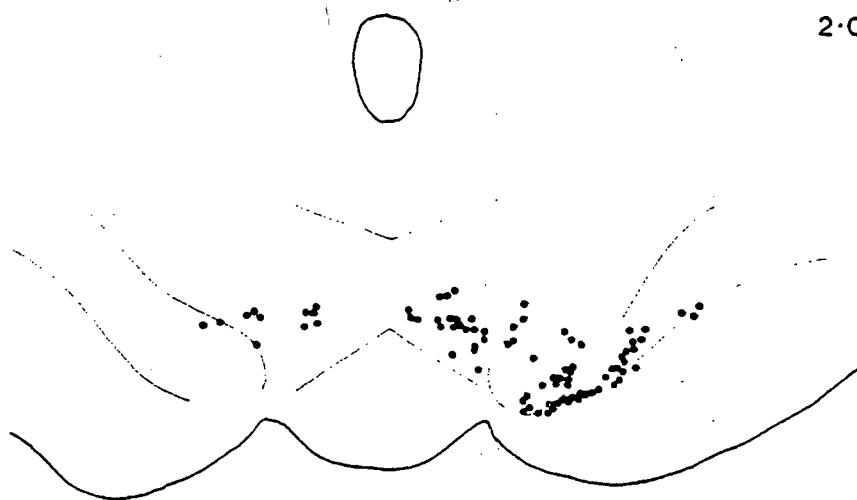


Fig. 4.8 continued.



4.3.4 RESTRICTED 6-OHDA LESIONS OF THE VENTRAL TEGMENTAL DECUSSATION.

6-OHDA (2ul, 4ug) lesions of the DTV were performed in 7 rats. These lesions used a 30 degree oblique cannula approach to the DTV in order to avoid damage to the overlying periaqueductal grey and dorsal raphe nuclei. It was considered important to minimise non-specific damage, and the intent of this exercise was a lesion specifically of the DTV. The lesioned rats showed increased locomotor activity post lesion. $7 \pm 4.3 / 27.5 \pm 4.4$ nett/total spontaneous rotations were performed on day 24, compared with $1.1 \pm 0.5 / 17 \pm 2.1$ rotations on day 1 pre-lesion (table 4.10). These values are not significantly different. This trend towards increased locomotor activity was continued with amphetamine (1mg/Kg, i.p.), with $16.6 \pm 9.4 / 98 \pm 13.4$ total rotations on day 24 compared with $6.4 \pm 2.0 / 28.3 \pm 2.6$ on day 1 pre-lesion (table 4.11). These values are significantly different (see table for details). There was a tendency to deviate from symmetrical behaviour towards the side from which the midline was approached by the cannula, as indicated by nett rotational performance. This is presumably due to the diffusion of neurotoxin along the cannula track displacing the mean lesion site 0.17mm from the midline towards the dominant ventral mesencephalon. The neurotoxin release zone was most often just dorsal to the central IP, and a trail of involuted cells followed in the cannula track through the ipsilateral VTN (see fig. 4.11). It is the damage to the ipsilateral VTN which probably caused this change in asymmetry. The data from 7 animals are

presented in table 4.8 and 4.9. Mean \pm SEM are graphically represented in fig. 4.9 and 4.10. The AI for spontaneous rotations was unchanged over the test period (table 4.12). For amphetamine (1mg/Kg, i.p.) induced rotations, AI on day 24 post lesion was significantly different from the first screen ($p=0.019$, table 4.13). This reflects the change in the animals' natural asymmetry to ipsilateral to the lesioned side post lesion. The lesion locations of this group are illustrated in fig. 4.11. The lesion mean \pm SEM is AP=2.4 \pm 0 LAT=0.17 \pm 0.03 D=9.33 \pm 0.05. There was no vacuolation of the tissue. Thus, the lesions were neurochemically specific for catecholamines.

It is concluded that lesioning of the DTV with 6-OHDA induces hyper-reactivity to amphetamine. This trend is also reflected for spontaneous behaviour, though the effect is small. This may in part be due to the limitations of the rotometer which is not optimised to measure motor hyperactivity. This concurs with the literature, notably Galey et al. (1977). The mesolimbic projection originates in part from the DAergic cells dorsal to the interpeduncular neurones. This A10 cell group has been implicated in locomotion, and its site of termination, n. Acc, in the activity component of circling (Pycock et al. 1978). Disruption of interhemispheric communication via the crossed projection may also be of functional importance in this observation, though it is unlikely (see discussion).

Table 4.10 The effect of restricted 6-OHDA les^oining of the DTV on spontaneous rotational behaviour. Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the side of cannula approach. S represents the pre-lesion screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion. n=7. The mean and SEM of this data are graphically represented in fig 4.9.

RAT	AP	LAT	D	S1	S2	7	14	24
154	2.4	0.4	9.4	-5.5/9	1/5.5	-5.5/18.5	1/31.5	-3.5/26
155	2.4	0.2	9.5	-3.5/8	1/3.5	9/24	1.5/5.5	5.5/11
156	2.4	0.2	9.6	-3.5/31.5	-11/14	-28/31.5	-9/16.5	-14.5/27.5
*157	2.4	0.5	9.6	3.5/42.5	7.5/18.5	5.5/9	4.5/5.5	1/3.5
(*)158	2.4	0.0	9.4	0.5/6	3.5/9	-7.5/24	1.5/26	5.5/10
187	2.4	0.0	8.6	-1/3	-0/5.5	26/48	40/102	40/94
195	2.4	0.1	9.2	-1.5/19.5	0.5/6	-1.5/48	-7.5/33.5	-1.5/20.5
Nett	2.4	0.17	9.33	-1.1	0.35	-0.28	4.5	7
+SEM	0	0.03	0.04	0.5	0.8	2.4	2.4	4.3
Total				17	8.8	29	31.5	27.5
+SEM				2.1	0.8	2.1	4.7	4.4

Rotational behaviour post-lesion not significantly different from pre-lesion behaviour, Mann Whitney U tests. Nett rotations, S1 vs day 24 $p > 0.1$. Total rotations, S1 vs day 24 $p = 0.228$.

FIG. 4.9. Schematic representation of the spontaneous rotational responses to restricted DTV lesions. Fig. of data presented in table 4.10. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total[^] rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. n=7. Total rotations show an increase in activity post lesion, though this is not significant. Nett rotations show there to be a slight rotational bias towards the side of cannula penetration. This is probably due to leakage of neurotoxin from the midline along the cannula track, affecting the DAergic cells of the ipsilateral VTN.

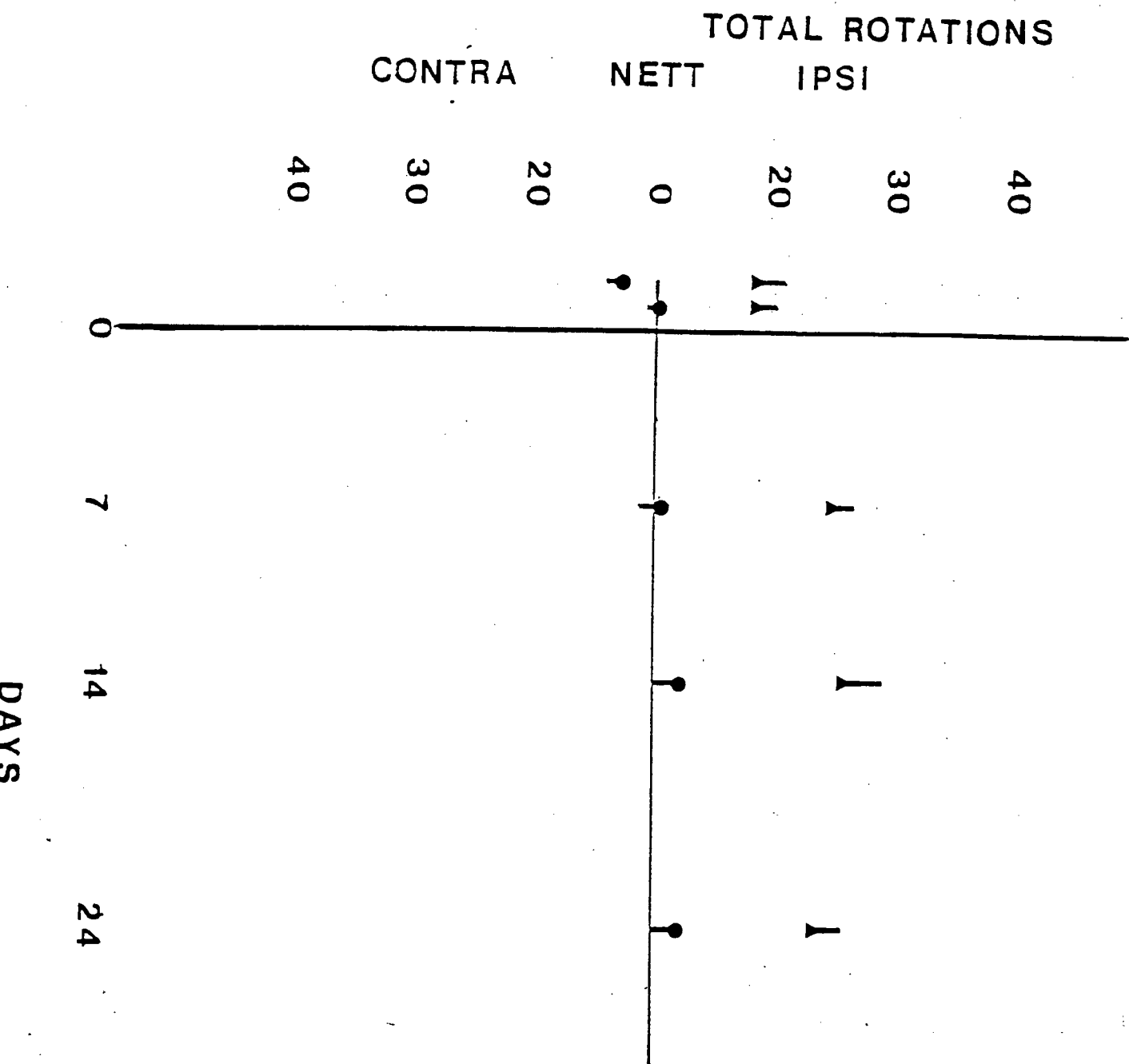


Table 4.11 The effect of restricted 6-OHDA lesioning of the DTV on amphetamine (1mg/Kg, i.p.) driven rotational behaviour (n=7). Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion. n=7. The mean and SEM of this data are graphically represented in fig 4.10.

RAT	AP	LAT	D	S1	S2	7	14	24
154	2.4	0.2	9.4	-38/44	-5/6	89/115	72/102	3/27
155	2.4	0.2	9.5	-3.5/5	-1.25/7.75	-2.5/30	-10/51	7/38
156	2.4	0.2	9.6	8/53	-44/55	-54/165	-77/92	-100/117
*157	2.4	0.5	9.6	4/46	49/137	19/22	27/31	23/28
158	2.4	0	9.4	-5.25/5.25	-17/19	-32/49	-20/63	+30/87
187	2.4	0	8.6	-5/25	-5/58	48/317	200/292	144/314
195	2.4	0.1	9.2	-5/20	1.75/7.75	1/78	-5/65	9/75
Nett	2.4	0.17	9.33	-6.4	-3.07	9.78	26.7	16.6
+SEM	0	0.03	0.05	2.0	3.6	6.4	11.75	9.4
Total				28.3	41.5	110.9	99.4	98
+SEM				2.6	6.3	13.7	11.6	13.4

Nett rotations on day 24 significantly different from S1 $p=0.036$.

Total rotations on day 24 significantly different from S1 $p=0.027$.

Mann Whitney U tests.

FIG. 4.10. Schematic representation of the rotational responses of animals with restricted DTV lesions to amphetamine (1mg/Kg, i.p.). Fig. of data presented in table 4.11. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=7$. The trends shown for spontaneous behaviour are mirrored for amphetamine-driven behaviour. There is an increase in total rotations, and nett rotations deviate towards the side of cannula penetration ($p=0.027$, $p=0.036$).

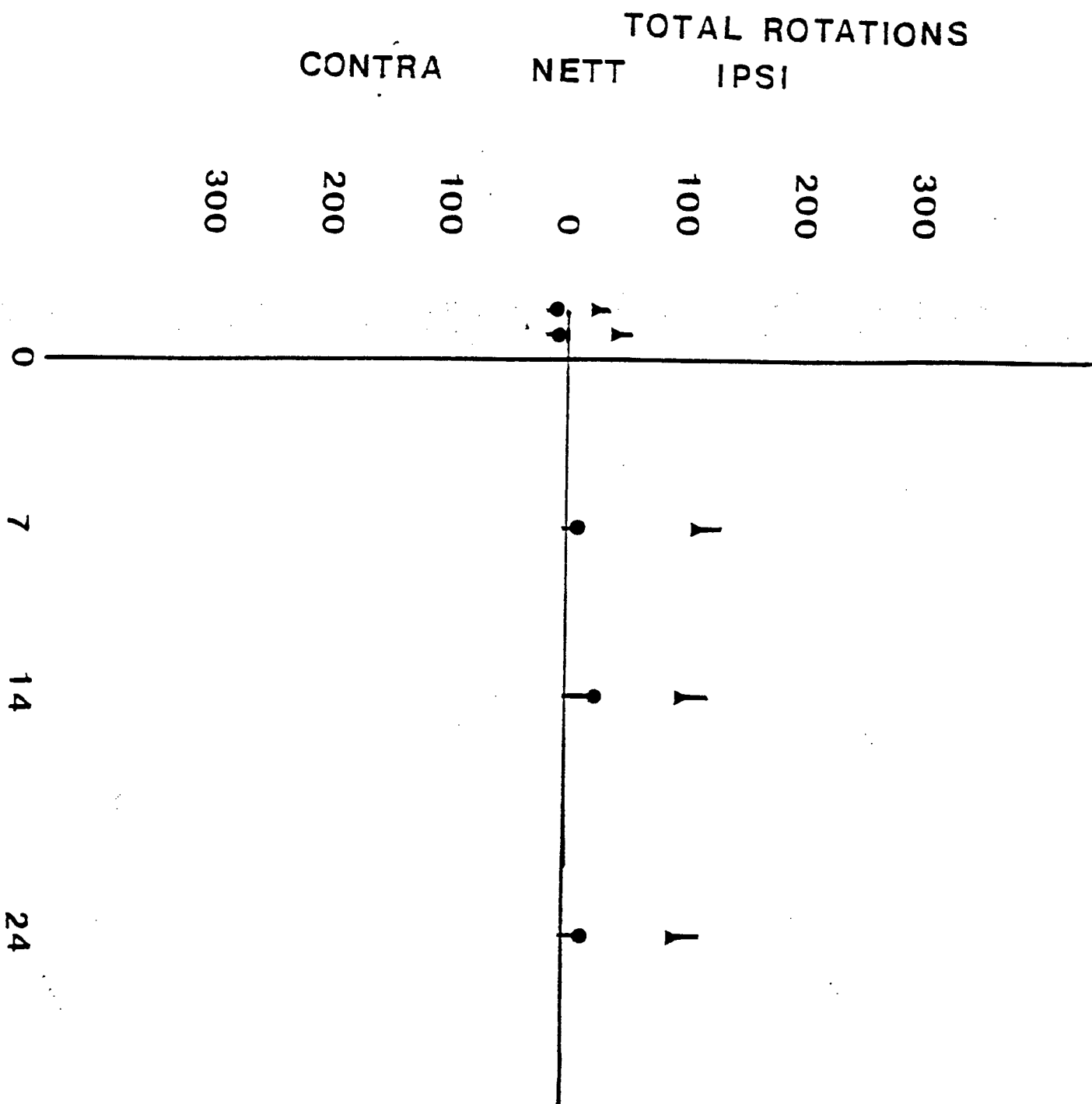


Table 4.12 Table of asymmetry index (Nett/Total*100) of spontaneous rotations with DTV lesion. S represents the screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion. n=7.

RAT	S1	S2	7	14	24
154	-52	17	-30	3	-16
155	-44	25	36	45	50
156	-12	-80	-86	-60	-53
157	9	40	67	83	25
158	8	37	-31	13	55
187	-43	0	53	41	43
195	-10	8	-7	-20	-7
Mean	-20.6	6.7	0.28	15	13.8
+--SEM	3.6	5.8	7.8	6.7	5.8

No significant differences.

Table 4.13 Table of asymmetry index (Nett/Total*100) of amphetamine (1mg/Kg, i.p.) driven rotations with DTV lesion. S represents the screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion. n=7.

RAT	S1	S2	7	14	24
154	-85	-83	78	71	11
155	-70	-16	-8	-19	19
156	15	-79	-94	-84	-86
157	8	36	84	85	81
158	-100	-89	-64	-31	23
187	-19	-9	15	68	46
195	-26	23	1	-8	12
Mean	-39.5	-31	1.7	11.7	15
+SEM	6.6	7.5	9.4	9.1	7.3

AI on day 24 significantly different from S1. $p=0.019$ Mann Whitney U test.

FIG. 4.11. Locations of restricted 6-OHDA lesions of the DTV, n=7. Sections conform to Pelligrino et al. (1979, AP=2.4). Grey areas show the extent of the lesion. Within these areas, cellular involution was present. The neurotoxin release zone was most often just dorsal to central IP, and a trail of involuted cells followed in the cannula track through the ipsilateral VTN. The black marking on the final diagram represents the mean lesion location for the 7 animals. DTV ventral tegmental decussation, IP interpeduncular nucleus, LM medial lemniscus, SN substantia nigra, VTN ventral tegmental nucleus of Tsai.

154

8

AP=2.4

155

156

157

158

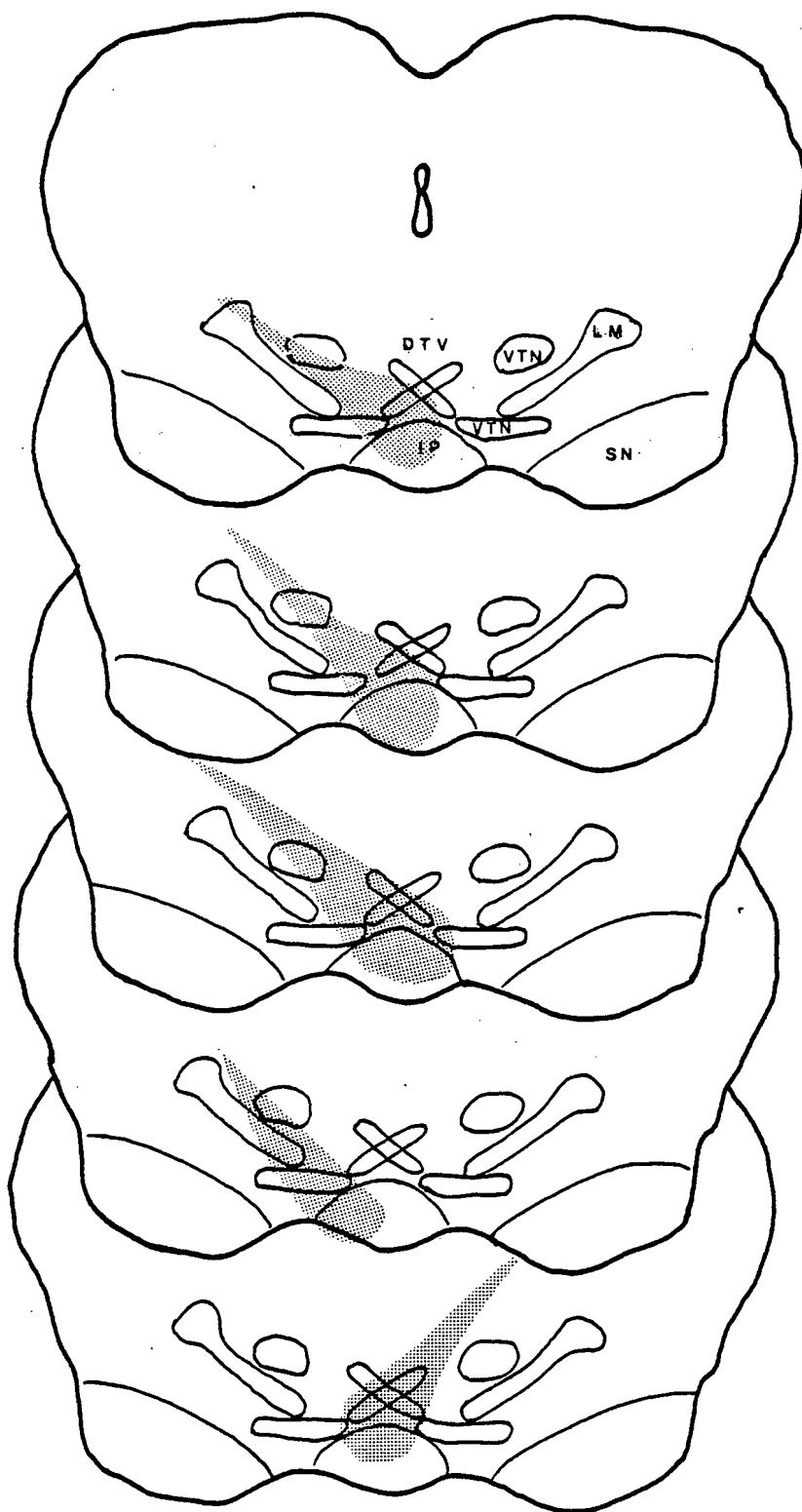
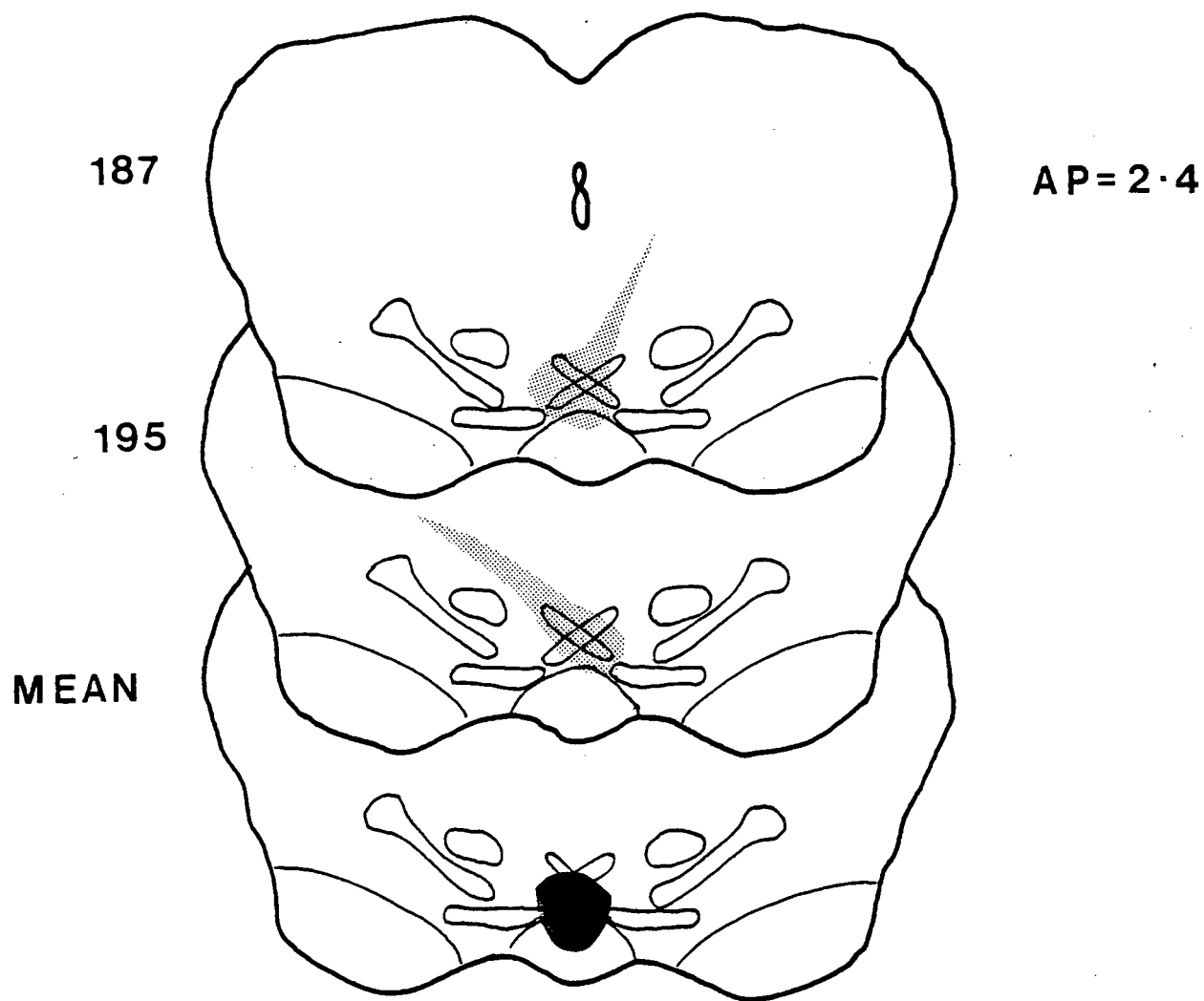


Fig. 4.11 continued.



4.3.5 RESTRICTED 6-OHDA LESIONS OF THE VENTRAL SN.

2ul 6-OHDA was infused into the ventral SN, AP=2.4 LAT=1.5 D=9.6. In 8 animals there was no crossing of rotation direction from the pre-lesion direction to that ipsilateral to the lesioned side. An increase in the number of nett and total rotations was noted. The data of 8 rats is summarised in tables 4.14 and 4.15. Spontaneous behaviour, expressed as nett/total rotations \pm SEM, was $16.3 \pm 2.5 / 57.4 \pm 9.0$ on day 24 compared with $0.4 \pm 0.7 / 15.5 \pm 1.4$ on day 1 pre-lesion. Amphetamine (1mg/Kg, i.p.) induced behaviour was $112 \pm 11 / 190.1 \pm 13.5$ on day 24 compared with $12.5 \pm 2.3 / 58.1 \pm 2.7$ on day 1 pre-lesion. There was thus a trend, both for spontaneous and amphetamine-driven rotational behaviour, towards an increase in total and nett contralateral rotations over the test period (fig. 4.12 and 4.13). This increase was statistically significant for nett and total rotations, both spontaneously and

with amphetamine (see tables for details). AI for spontaneous rotations was unchanged over the test period (table 4.16). The AI for amphetamine (1mg/Kg, i.p.) induced rotations was unchanged over the test period (table 4.17). Thus, ventral SN lesions do not affect asymmetry; they only induce spontaneous and amphetamine-induced hyperactivity. The lesion locations are illustrated in fig. 4.14. There was no vacuolation of the ventral SN, and little involution of cells in the region of neurotoxin deposition other than that caused by the cannula track. This is due to the scarcity of DAergic cells within the ventral SN.

It is concluded that there is no effect of 6-OHDA lesioning of the ventral SN on inducing rotational behaviour ipsilateral to the lesioned side. Locomotor hyperactivity was observed in response to the lesion, both spontaneously and with amphetamine. This specific hyperactivity response may be part of the behavioural syndrome observed with standard 6-OHDA lesions of the SN, in which the ventral SN is often affected. The reason for the hyperactivity can only be speculated about on the basis of this data. It may involve negation of the auto-inhibitory effect of nigral dopamine mediated by dendritic dopamine receptors within the ventral SN (see discussion).

Table 4.14 The effect of restricted 6-OHDA lesioning of the ventral SN on spontaneous rotational behaviour. Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion. n=8. The mean and SEM of this data are presented graphically in fig. 4.12.

RAT	AP	LAT	D	S1	S2	7	14	24
124	2.4	1.8	9.6	-6.5/10	-5.5/15	-1.5/17	3.5/13	-30/63
125	2.4	1.5	9.6	-1.5/17	11/24	9/48	-2.5/35.5	13/57.5
28	2.4	2.5	9.6	1/1	1/3.5	-24/30	-20/29	-18.5/33.5
130	2.4	0.8	9.6	-2.5/31.5	0	6/58	1.5/41	1.5/1.5
132	2.4	1.0	9.6	3.5/20.5	2.5/3.5	-0.5/26	-1.5/122	-58/228
133	2.4	1.2	9.6	7.5/28	-0.5/21	5-5/39	-15/30	-14/46
170	2.4	1.0	9.6	-0/0	-5.5/8	-0.5/7	-8/8	-12.5/17
181	2.4	1.0	9.6	1.5/16	-1.5/5.5	-7.5/12	-0/1	-13/13
Nett	2.4	1.35	9.6	0.4	0.18	-1.6	-5.25	-16.3
+--SEM	0	0.07	0.5	0.7	1.3	1.1	2.7	2.5
Total				15.5	10	29.6	34.9	57.4
+--SEM				1.4	1.1	2.2	4.7	9.0

Nett rotations on day 24 post-lesion significantly different from S1, $p < 0.032$.

Total rotations on day 24 post-lesion significantly different from S1, $p < 0.041$.

Mann Whitney U tests.

FIG. 4.12. Schematic representation of the spontaneous rotational responses to restricted ventral SN lesions. Fig. of data presented in table 4.14. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=8$. There is no change of nett rotation direction from contralateral to ipsilateral to the lesioned side post lesion, though there is a trend towards an increase in total and nett contralateral rotations over the test period ($p<0.041$, $p<0.032$).

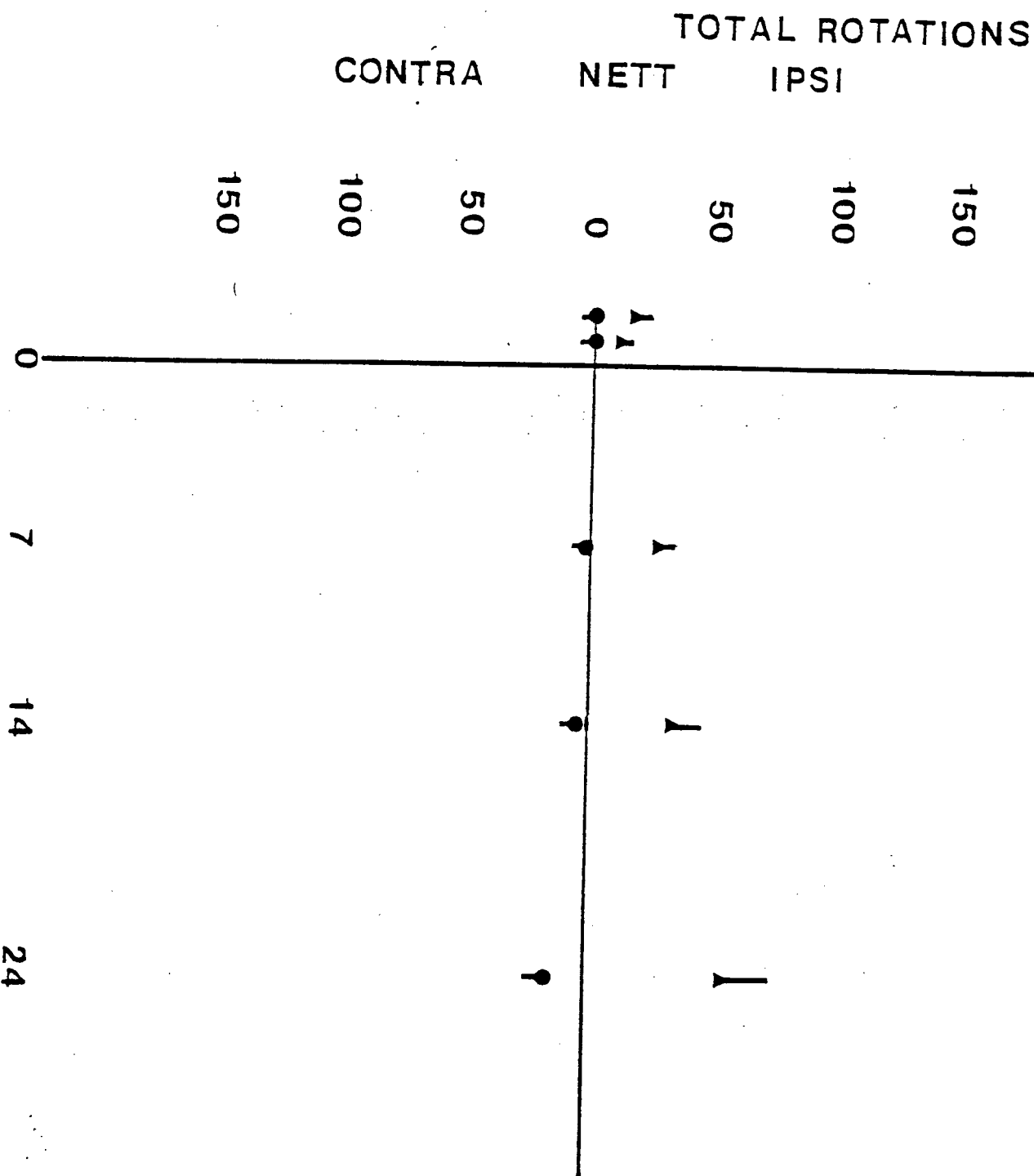


Table 4.15 The effect of restricted 6-OHDA lesioning of the ventral SN on amphetamine (1mg/Kg i.p.) driven rotational behaviour. Data is presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion. n=8. The mean and SEM of this data are presented graphically in fig 4.13.

RAT	AP	LAT	D	S1	S2	7	14	24
124	2.4	1.8	9.6	-0.5/79	-83/95	-111/208	-189/222	-150/198
125	2.4	1.5	9.6	6.5/75	32/101	21/96	15/207	13/122
128	2.4	2.5	9.6	-32/49	-9/15	-156/164	-177/191	-210/227
130	2.4	0.8	9.6	-8/60	-7/33	55/343	-3/61	15/26
132	2.4	1.0	9.6	1/47	-31/50	-56/301	-48/214	-76/401
133	2.4	1.2	9.6	-20/87	-26/62	-145/205	-186/253	-169/224
170	2.4	1.0	9.6	-45/48	-75/80	-127/80	-120/186	-133/135
181	2.4	1.0	9.6	-2/20	-19/33	-197/197	-116/117	-186/188
Nett	2.4	1.4	9.6	-12.5	-27.2	-89	-107	-112
+SEM	0	0.07	0	2.3	4.7	11.1	9.6	11
Total				58.1	58.6	205	181.4	190.1
+SEM				2.7	3.9	10.3	7.8	13.5

Nett rotations on day 24 post-lesion significantly different from S1, $p=0.052$.

Total rotations on day 24 post-lesion significantly different from S1, $p=0.003$.

Mann Whitney U Tests.

FIG. 4.13. Schematic representation of the rotational responses of animals with restricted ventral SN lesions to amphetamine (1mg/Kg). Fig. of data presented in table 4.15. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Total rotations are shown above the x-axis. Data are mean and SEM. $n=8$. There is no change in nett rotational direction from contralateral to ipsilateral to the lesioned side post lesion, though a trend towards an increase in total and nett contralateral rotations is apparent over the test period ($p<0.003$, $p<0.052$).

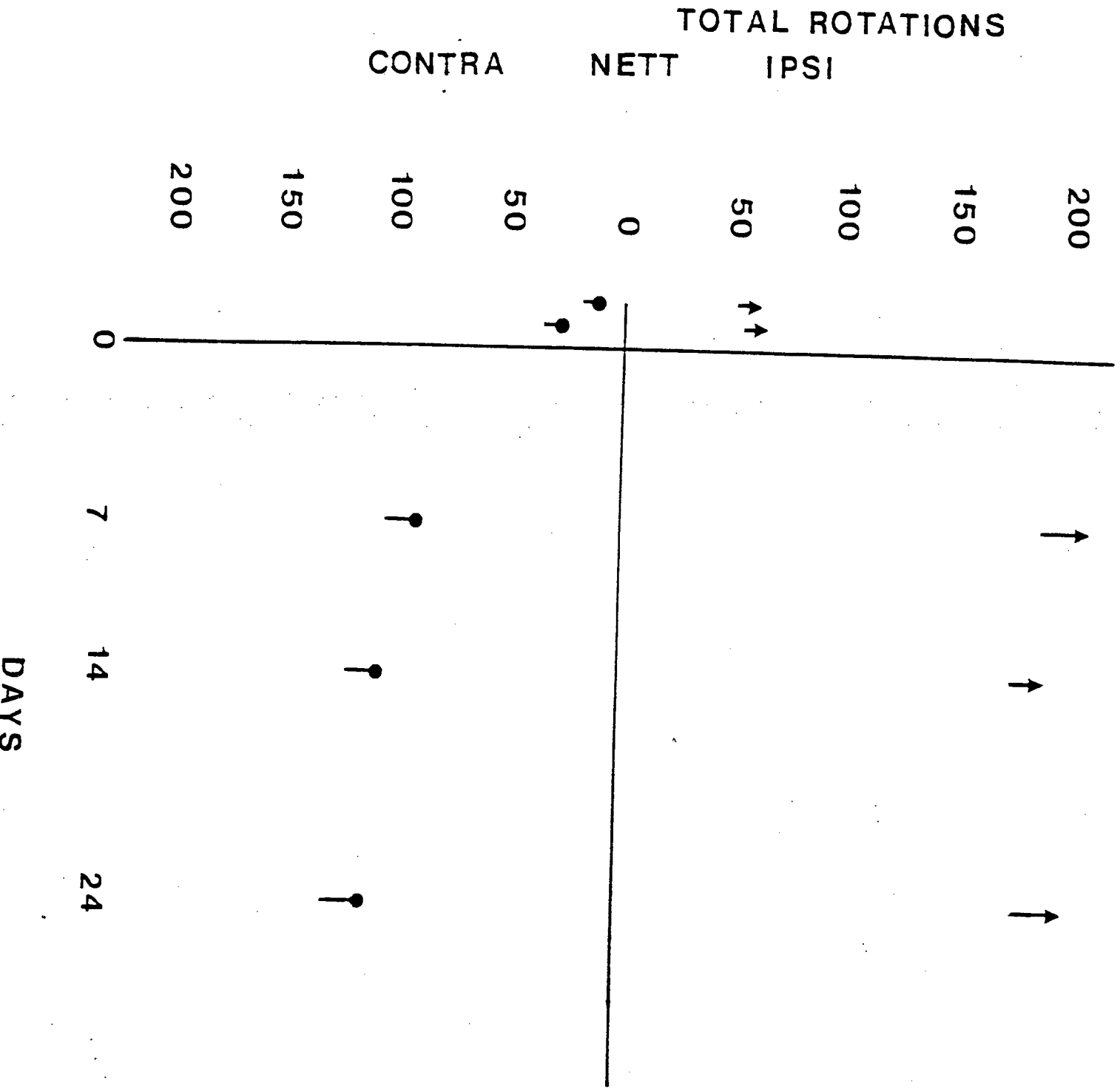


Table 4.16 Table of asymmetry index (Nett/Total*100) of spontaneous rotations with ventral SN lesions. S represents the screens, while 7, 14 and 24 are the days on which behaviour was tested post-lesion.

RAT	S1	S2	7	14	24
124	64	-35	-8	26	48
125	-4	44	20	6	22
128	100	25	-81	-76	-52
130	-9	0	10	3	100
132	-14	75	-2	-2	-25
133	28	-2	14	-50	-30
170	0	-67	-7	-100	-73
181	12	-33	-62	0	-94
Mean	22.1	0.8	-14.5	-24.1	-13
+SEM	5	5.8	4.6	5.7	8.2

No significant differences.

Table 4.17 Table of asymmetry index (Nett/Total*100) of amphetamine (1mg/Kg, i.p.) driven rotations with ventral SN lesions. S represents the screens while 7, 14 and 24 are the days on which behaviour was tested post-lesion.

RAT	S1	S2	7	14	24
124	-1	-87	-53	-85	-76
125	9	31	22	7	11
128	-65	-61	-95	-93	-93
130	-13	-20	16	-5	56
132	2	-63	-19	-23	-19
133	-23	-42	-71	-73	-75
170	-93	-94	-98	-65	-99
181	-10	-58	-100	-99	-99
Mean	-24	-49.3	-49.7	-54.5	-49.3
+--SEM	4.5	5	6.3	5.2	7.3

No significant differences.

FIG. 4.14. Locations of restricted 6-OHDA lesions of the ventral SN. Sections conform to Pelligrino et al. (1979, AP=2.4). There was no vacuolation present and little involution of cells in the region of neurotoxin deposition. Involuting cells were present in the vicinity of the cannula track. The extent of the lesions is indicated by stippling. DTV ventral tegmental decussation, IP interpeduncular nucleus, LM medial lemniscus, VTN ventral tegmental nucleus of Tsai.

124

AP = 2.4

125

128

130

132

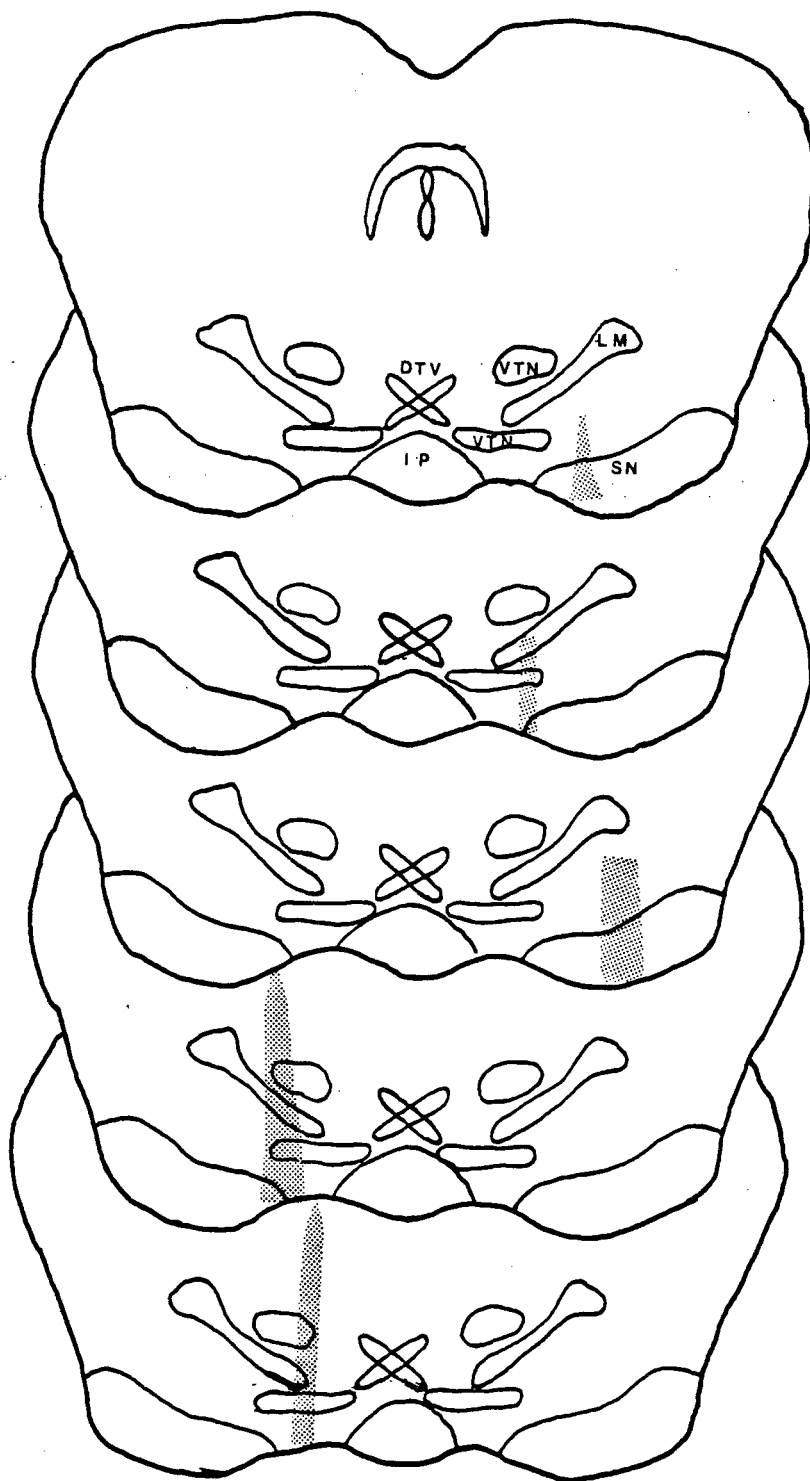


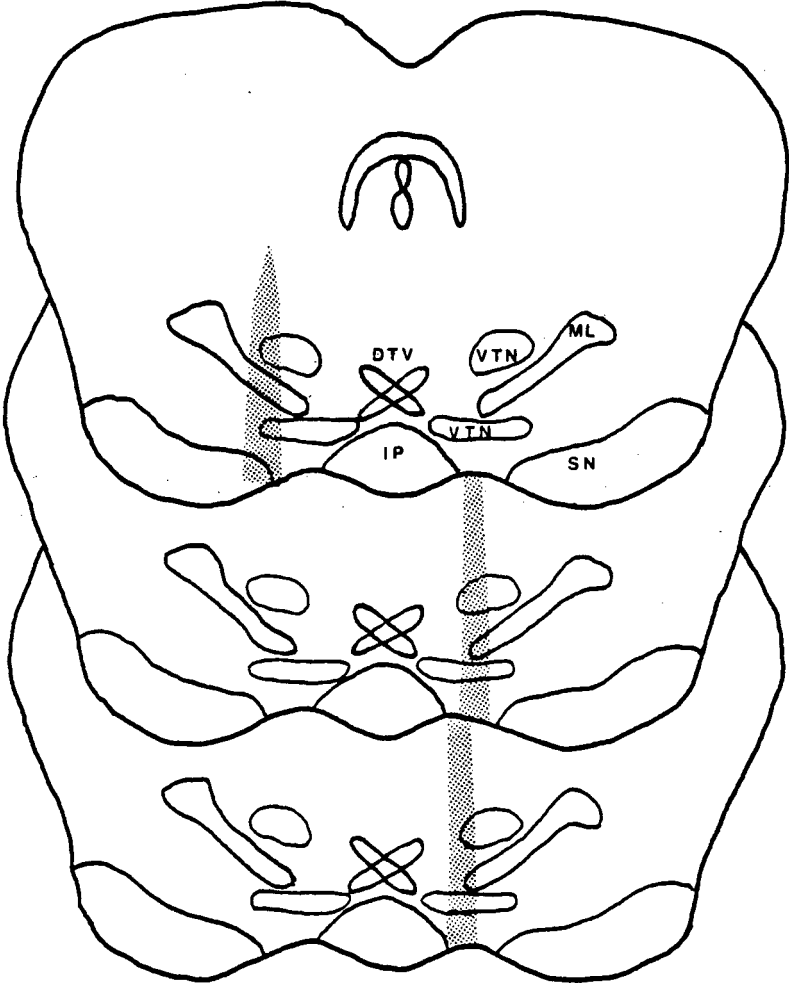
Fig. 4.14 continued.

133

AP=2.4

170

181



4.3.6 RESTRICTED 6-OHDA LESIONS OF THE ANTEROMEDIAL SN.

Recovery from sensory-motor asymmetry is apparent from the work of Glick and Cox (1978), and more recently by Pritzel et al. (1983) and Dravid et al. (1984). The criterion used to determine "recovery from sensory-motor asymmetry" was in both cases, an attenuation of nett ipsilateral rotations on the final day of testing compared with the first day. Glick and Cox (1978) demonstrated recovery from 110.1 ± 12.1 to 74.1 ± 4.2 turns per hour (32.6%) 35-36 days later, in animals with electrolytic lesions of the dominant SN. Costall et al. (1976) found no attenuation of amphetamine induced rotation in response to 6-OHDA lesioning of the SN, but only measured behaviour for 18 post-operative days. Pritzel et al. (1983) used recovery from spontaneous rotational behaviour as their determinant of sensory-motor rehabilitation. They also tested with amphetamine, but did not report numbers of rotations, simply observing whether or not ipsilateral rotation was inducible at various days post lesion. Circling varied between 20 and 100 spontaneous ipsilateral turns per hour for the first four days post lesion. Thereafter, until 90 days, circling was elicited as part of the startle reaction. Dravid et al. (1984) measured amphetamine (5mg/Kg) and apomorphine (1mg/Kg) induced rotation in rats lesioned with 1, 2, and 8ug 6-OHDA in the SN. Behaviour was recorded from 7 to 80 days. They found that rats with partial (1 or 2ul) lesions of the SN exhibited a significant

decrease in amphetamine-induced circling 22 days after lesioning, and at subsequent periods. This decrease amounted to a mean circling rate 25-30% of that at day 7 post lesion. There was no response to apomorphine. Their lesioning site (AP2.42, LAT 1.6, V-2.6. Koenig and Klippel 1963) was more lateral and caudal than those employed in this study (AP=3.0 LAT=1.0 D=7.4 (Pelligrino et al. 1979). Animals lesioned with large doses of 6-OHDA (4ul, 8ug) did not exhibit recovery from nett ipsilateral rotations (Dravid et al. 1984).

32 male Long-Evans rats (270-350g) were used in this study. They were housed in cages of 8 rats, with ad libitum food and water. Amphetamine (1mg/Kg, i.p.) was administered pre-lesion on two consecutive days in order to determine the hemisphere dominant for rotation. The restricted 6-OHDA lesion of the anteromedial SN was performed on the third day, and behavioural testing followed on days 7, 14, 24 and 32 post lesion. Behavioural monitoring was via the automated procedure described in chapter 2. The number of rotations on day 32 was contrasted with that on day 7 in order to gauge the animal's state of recovery from the lesion. Recovery was determined by decreased nett ipsilateral rotations on the final day of testing compared with the first. This is the protocol encountered in the literature. In addition to assessing nett ipsilateral rotations, total rotations and AI were also assessed for recovery. This enabled the discrimination between recovery from asymmetry and hyperactivity. Prior to each session in the rotometer, the animal's orientation to its environment was tested by presenting various stimuli viz; (a) noxious olfactory (acetic

acid soaked cotton bud), (b) tactile (pinch, whisker tweaking), and (c) auditory (finger snap close to ear). The stimuli were presented ipsi- and contralaterally to the lesioned side in order to establish a qualitative index of the subjects sensory-motor responsiveness in the presence of a lesion. The results of these tests are presented in appendix 4.

The extent of the 6-OHDA lesion was determined by evaluation of the HRP labelling of lesioned SN' compared to the unlesioned controls established in the previous section (254-277). Cresyl fast violet stained sections of the lesion site were also inspected (94-230), fig. 4.23.

The 32 animals used in this study were classified into 4 categories based on their nett ipsilateral rotational performance. 1) Spontaneous recoverers 2) Amphetamine recoverers 3) Spontaneous non-recoverers 4) Amphetamine non-recoverers. A decrease in the number of rotations on day 32 post lesion compared to day 7 was taken to indicate recovery, while no change or an increase indicated non-recovery.

Table 4.18 shows the responses of 18 of 32 animals fulfilling the criterion for recovery from spontaneous rotation. Nett ipsilateral spontaneous rotations decreased from 18 ± 2 on day 7 post lesion to 7.2 ± 0.4 on day 32 post lesion (mean \pm SEM), $p < 0.01$ Mann Whitney U test. The mean and SEM of nett and total rotations are

illustrated for each day tested in fig. 4.15, 4.16 respectively.

Recovery from amphetamine (1mg/Kg, i.p.) induced rotation was noted in 14 animals, decreasing from 262 ± 13 to 143 ± 7.8 , $p < 0.01$ Mann Whitney U test (table 4.20). The mean and SEM of nett and total rotations are illustrated in figs. 4.18, 4.19 respectively.

14 of 32 animals did not recover from spontaneous rotations, (table 4.22). The mean and SEM of the data are graphically represented in fig. 4.21. 18 rats did not recover from amphetamine induced rotations, table 4.24, fig. 4.22.

Recovery was not necessarily from both spontaneous and amphetamine induced rotation for a given animal. Thus 7 animals (94, 95, 254, 258, 260, 261, 276) recovered from both spontaneous and amphetamine induced behaviour, 11 (100, 103, 225, 228, 230, 255, 256, 257, 259, 272, 273) recovered from spontaneous rotation only, 7 (113, 139, 229, 263, 270, 271, 275) recovered from amphetamine induced rotation only. 7 animals (98, 108, 137, 262, 264, 265, 277) showed no recovery or a potentiation of nett rotation in both instances, 7 (113, 139, 229, 263, 270, 271, 275) displayed no recovery from spontaneous behaviour only, and 11 (100, 103, 225, 228, 230, 255, 256, 257, 259, 272, 273) did not recover from amphetamine-driven behaviour only.

Table 4.14 and fig. 4.17 illustrate the asymmetry index of the group of animals defined as recoverers from spontaneous nett ipsilateral rotations (table 4.14, fig. 4.15, 4.16). The index was

statistically unchanged on day 32 ($53.3 \pm 1.3\%$) as compared to day 7 ($51 \pm 1.7\%$). The group showing recovery from amphetamine driven rotation (table 4.20, figs. 4.18, 4.19) had an asymmetry index of $88 \pm 10.7\%$ on the final test (day 32) as compared to $84 \pm 18.6\%$ on the first test post lesion (day 7). These values do not differ significantly. Thus, recovery was from motor hyperactivity rather than motor asymmetry. (Fig 4.20 Table 4.21).

The tests for sensory-motor rehabilitation revealed that lesioned animals remained impaired for the duration of the test period. These tests are detailed in appendix 4. The lesioned animals noticed stimuli presented ipsilaterally to the lesioned side. Their behaviour was, however, inappropriate following the lesion. The noxious olfactory stimulus was turned into, rather than away from. Likewise, lesioned rats were unable to escape noxious tactile stimuli presented ipsilaterally to the lesioned side. It was concluded that the 6-OHDA lesion affected the animal's ability to conduct appropriate behaviour when this meant moving contralaterally to the lesioned side. There was thus a definite motor impairment shown by these tests. Stimuli presented contralaterally to the lesioned side were often neglected. The animal did not elicit an ipsilateral escape from a contralaterally presented noxious olfactory or tactile stimulus. Similarly, it ignored finger snapping presented to the contralateral ear, while responding to the same stimulus presented ipsilaterally by ear twitching, head turning, or locomotion. The lack of attention to stimuli presented contralaterally suggests a sensory deficit following 6-OHDA lesioning of the anteromedial SN. In some animals

there was improvement of the sensory neglect and locomotor impairment in that whereas 24 hours after the lesion the animal was unresponsive to contralaterally presented stimuli, after 32 days it would show head turning in the direction of the stimulus. Noxious stimuli, however, continued to elicit inappropriate responses, and there were no animals recovering the ability to rotate contralaterally in order to "persue" a stimulus.

The locations of the lesions of the anteromedial SN are presented in fig. 23. Numbers 94 to 230 are schematic diagrams based on the atlas of Pelligrino et al. (1979). 254 to 277 are camera lucida drawings of ventral mesencephali, showing the distribution of HRP filled somata, retrogradely labelled from striatal deposition sites. The deposition site is included in the histological description, and had a consistently ventromedial placement. The mean \pm SEM of the focal point of the lesion locations for the various groups was obtained from histological examination. These are contrasted in the table below. SR spontaneous recoverers, NSR spontaneous non-recoverers, AR amphetamine recoverers, NAR amphetamine non-recoverers.

GROUP	AP	LAT	D	p VALUE recovered vs non-recovered
SR	3.17	1.08	7.8	
+-SEM	0.02	0.02	0.03	
NSR	3.06	1.02	8.16	p>0.1
+-SEM	0.02	0.01	0.06	
AR	3.12	1.09	7.9	
+-SEM	0.04	0.01	0.06	
NAR	3.08	1.0	8.16	p>0.1
+-SEM	0.02	0.02	0.06	

There was no significant difference between the mean lesion location of recoverers as compared to non-recoverers, either for spontaneous or amphetamine induced behaviour. These results suggest that the incidence of the behavioural recovery is not a function of the location of the lesion.

The HRP evaluation of lesioned ventral mesencephali revealed greatly attenuated cell counts when compared to controls. Labelled cells were reduced within the entire ventral mesencephalon, ipsilaterally as well as contralaterally. While the site of neurotoxin action could be seen within the anteromedial SNC and VTN, labelling of cells was reduced up to 2mm caudal and lateral to the lesion site. The lesions were sufficiently anterior so as to lesion the MFB. The anteriorly coursing fibres of SNC cells caudal and lateral to the diffusional spread of neurotoxin would have been lesioned, resulting in their retrograde degeneration. The axons of the crossed projection had decussated caudally to the lesion location, and they were thus also lesioned, resulting in

retrograde degeneration of cells in the contralateral ventral mesencephalon. The histological descriptions of the animals in this section are presented in fig. 4.23.

It is concluded that restricted 6-OHDA lesioning of the anteromedial SN induces crossing of rotational direction from the pre-lesion direction to that ipsilateral to the lesioned side for both spontaneous and amphetamine-driven rotations. The intensity of rotation was similar to that induced by standard lesions centrally placed within the SN. There is a differential recovery from the effects of the lesion when gauged by decreased nett ipsilateral rotations. The asymmetry index of the animals recovering from spontaneous nett rotations showed no change over the test period. The same trend holds true for amphetamine-induced rotations. These data suggest that the animals were recovering from locomotor hyperactivity rather than motor asymmetry. The type of recovery from restricted lesions of the anteromedial SN is similar to that observed from standard lesions of the SN.

Table 4.18 Animals exhibiting recovery from spontaneous rotations with restricted 6-OHDA lesion of the antero medial SN. Data presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14, 24 and 32 are the days on which behaviour was tested post-lesion. * indicates animal lesioned in the non-dominant hemisphere. n=18.

RAT	AP	LAT	D	S1	S2	7	14	24	32
94	3.0	0.8	8.5	-1.5/4	0/0		8.5/9	8/10.5	3/5
95	3.0	1.0	8.6	0/3.5	9/16.5		6.5/9	1.5/1.5	2/4
100	3.0	0.8	9.1	5/7	3/16		10/10	5/7	3/9
103	2.4	1.3	9.5	0.5/2.5	0/0	3/3	6/8	1.5/2.5	
225	3.8	1.6	8.6	0/0	4.5/4.5		2.5/8.5	1.5/2.5	0/0
228	3.0	1.2	8.8	-6.5/12	-8/16		44.5/46	33/41	20/26
230	3.0	1.3	8.6	1.5/46.5	0/16		33.5/44.5	11/16.5	12/14.5
254	3.6	1.0	7.4	2/13	-0.5/11	80/81	83/84	29/32	4/6
255	3.2	1.0	7.2	-13/36	-12/64	-20/49	14/30	7/14	4/7
256	3.0	1.0	7.4	5/19	7.5/42	16/32	4/18	5/13	5/9
257	3.0	0.5	7.4	1.5/3.5	0.5/80	5/39	3/36	0/21	2/18
*258	3.4	1.4	7.5	7/12	-4/43	4/38	27/28	9.5/10	3.5/6
259	3.2	1.2	7.6	40/116	39/145	38/81	21/34	38/41	22/28
260	2.6	1.4	7.4	-37/71	-14/50	17/41	7/21	22/33	6/17
261	2.8	1.2	7.4	0.75/0.753/17		7/10	10/13	10/21	5/6
272	3.4	0.8	7.2	3/10	1/24	32/42	24/28	13/26	19/35
273	3.0	1.0	8.1	2/37	-15/64	15/61	-2/17	7/24	8/16
276	3.8	1.0	7.4	-3/27	-2/9	16/41	3/12	2/5	4/12
Nett	3.12	1.08	7.98	0.7	0.9	18	17	11.3	7.2
+SEM	0.02	0.01	0.04	0.8	0.7	2	1.1	0.6	0.4
Total				23.4	37.6	43	25.3	17.8	12.8
+SD				1.7	2.1	2	1.1	0.7	0.6

Nett rotations on day 32 significantly different from day 7, $p < 0.05$.

Total rotations on day 32 significantly different from day 7, $p < 0.002$.

Nett rotations on day 32 significantly different from S1, $p < 0.02$.

Total rotations on day 32 not significantly different from S1, $p > 0.1$.

Total rotations on day 7 significantly different from S1, $p < 0.02$.

Mann Whitney U tests.

FIG. 4.15. Schematic representation of spontaneous rotational responses to restricted anteromedial SNC lesion. Fig. of data presented in table 4.18, showing animals recovering from the lesion induced ipsilateral rotation. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. The closed circles represent nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Data are mean and SEM. $n=18$. Animals show crossing of nett rotational direction on day 7 post lesion, from contralateral to ipsilateral to the lesioned side. There is a trend towards a decrease in the number of nett ipsilateral rotations over the test period ($p<0.05$).

ROTATIONS
CONTRA NETT IPSI

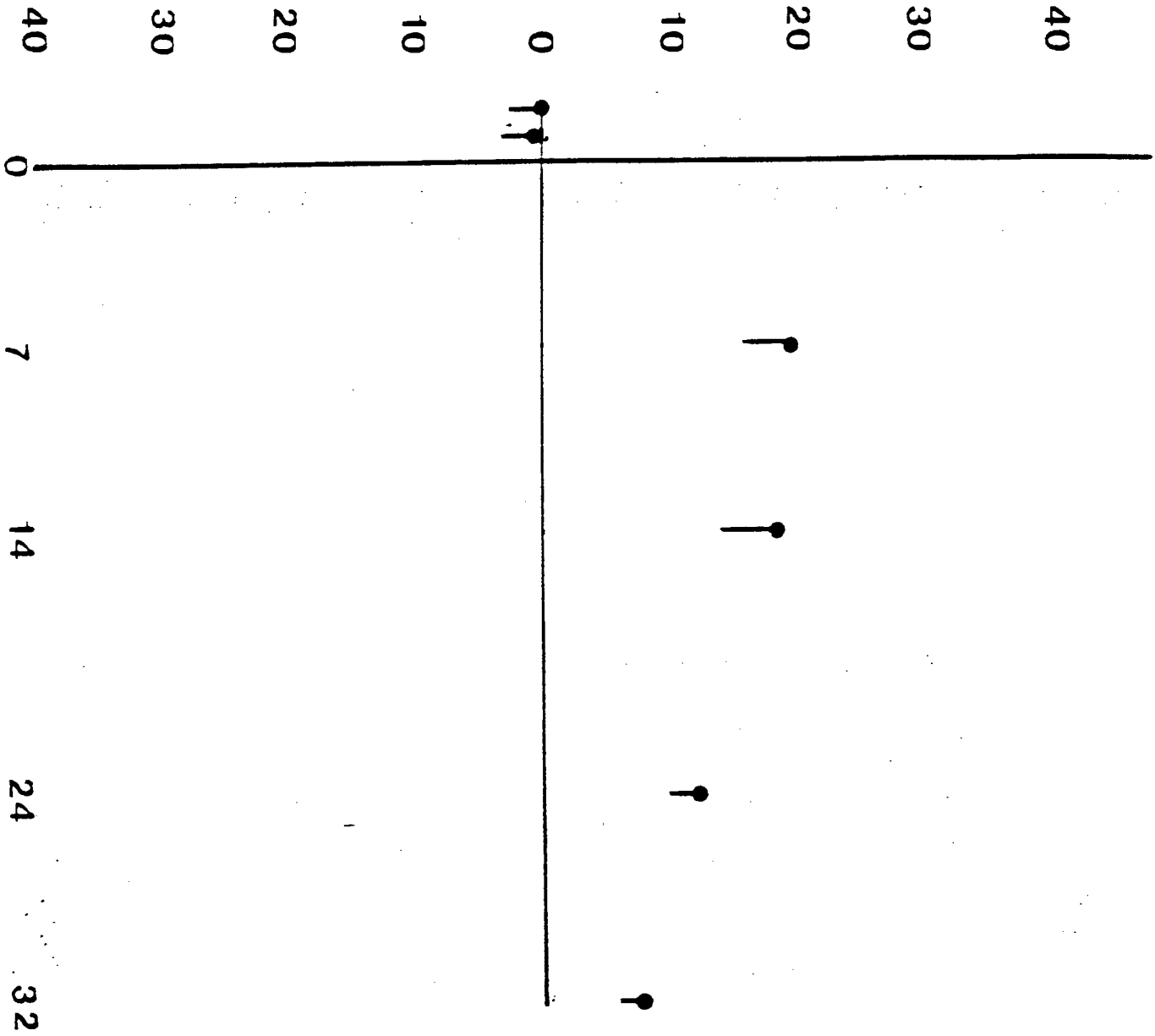


FIG. 4.16. Schematic representation of spontaneous rotational responses to restricted anteromedial SN lesion. Fig of data presented in table 4.18, showing animals recovering from the lesion induced ipsilateral rotation. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. The triangles represent total rotations. Data are mean and SEM. $n=18$. There is a trend towards a decrease in the number of total rotations over the test period ($p<0.002$).

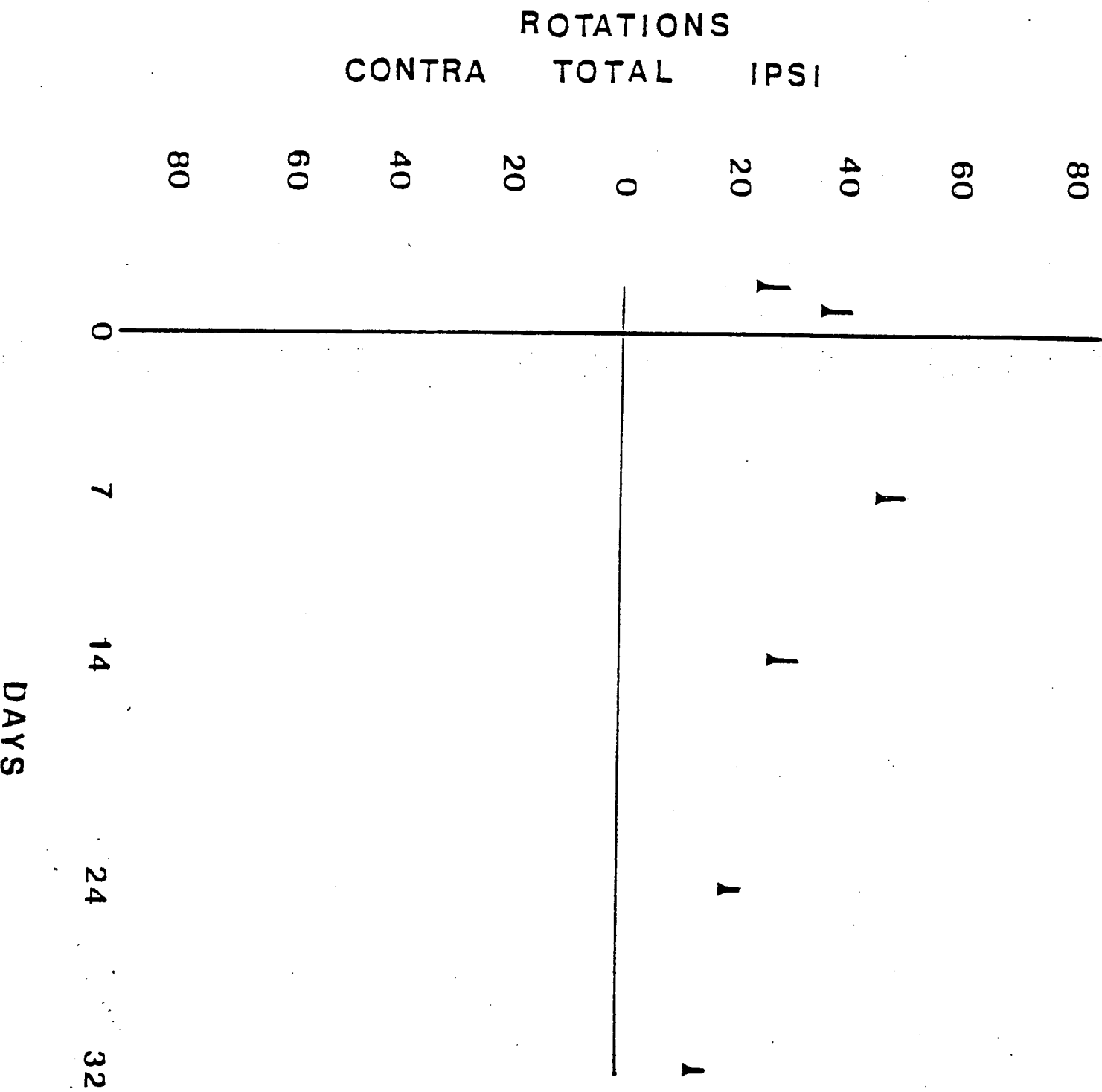


Table 4.19 Asymmetry index of animals exhibiting recovery as specified by a decrease in spontaneous nett ipsilateral rotations on the final test as compared to the first test. Spontaneous rotations expressed as $\text{Nett/Total} \times 100$. Positive values are ipsilateral to the lesioned side, negative contralateral. DAI is the change in the asymmetry index.; +ve indicating an increase in asymmetry, -ve a decrease. $n=18$.

RAT	S1	S2	7	14	24	32	DAI
94	-33	0		+90	+74	+64	-20
95	0	56		+70	+100	+50	-20
100	+73	-18		+100	+73	+24	-76
103	+20	0		+100	+76	+60	-40
225	0	+100		+33	+67	0	-33
228	-54	-49		+94	+82	+79	-15
230	+3	0	+74	+71	+81	+7	
254	+15	-4	+99	+98	+92	+80	-19
255	-41	+40	+47	+47	+47	+50	+10
256	+26	+18	+50	+23	+38	+60	+10
257	+27	+1	+12	+8	0	+11	-1
258	+58	-15	+11	+98	+95	+58	+47
259	+33	+24	+47	+62	+92	+77	+30
260	-51	-28	+41	+34	+66	+36	-8
261	-33	+21	+74	+70	+49	+76	+2
272	+32	+2	+75	+86	+50	+55	-20
273	+5	-24	+25	-12	+31	+50	+25
276	-10	-22	+38	+58	+60	+55	+17
Mean	+28.5	+22.3	+51	+62.9	+63.7	+53.3	-5.7
+SEM	1.2	1.4	1.7	1.7	1.4	1.3	1.6

AI on day 32 significantly different from S1, $p < 0.002$.

AI on day 7 significantly different from S1, $p = 0.002$.

AI on day 32 not significantly different from day 7, $p > 0.1$.

Mann Whitney U tests.

FIG. 4.17. Asymmetry index ($AI = \text{Nett} / \text{Total} * 100$) of animals exhibiting recovery from anteromedial SN lesion as shown by decreased spontaneous nett rotations. Fig. of data presented in table 4.19. Data are mean and SEM. $n=18$. There is no change in the AI over the test period.

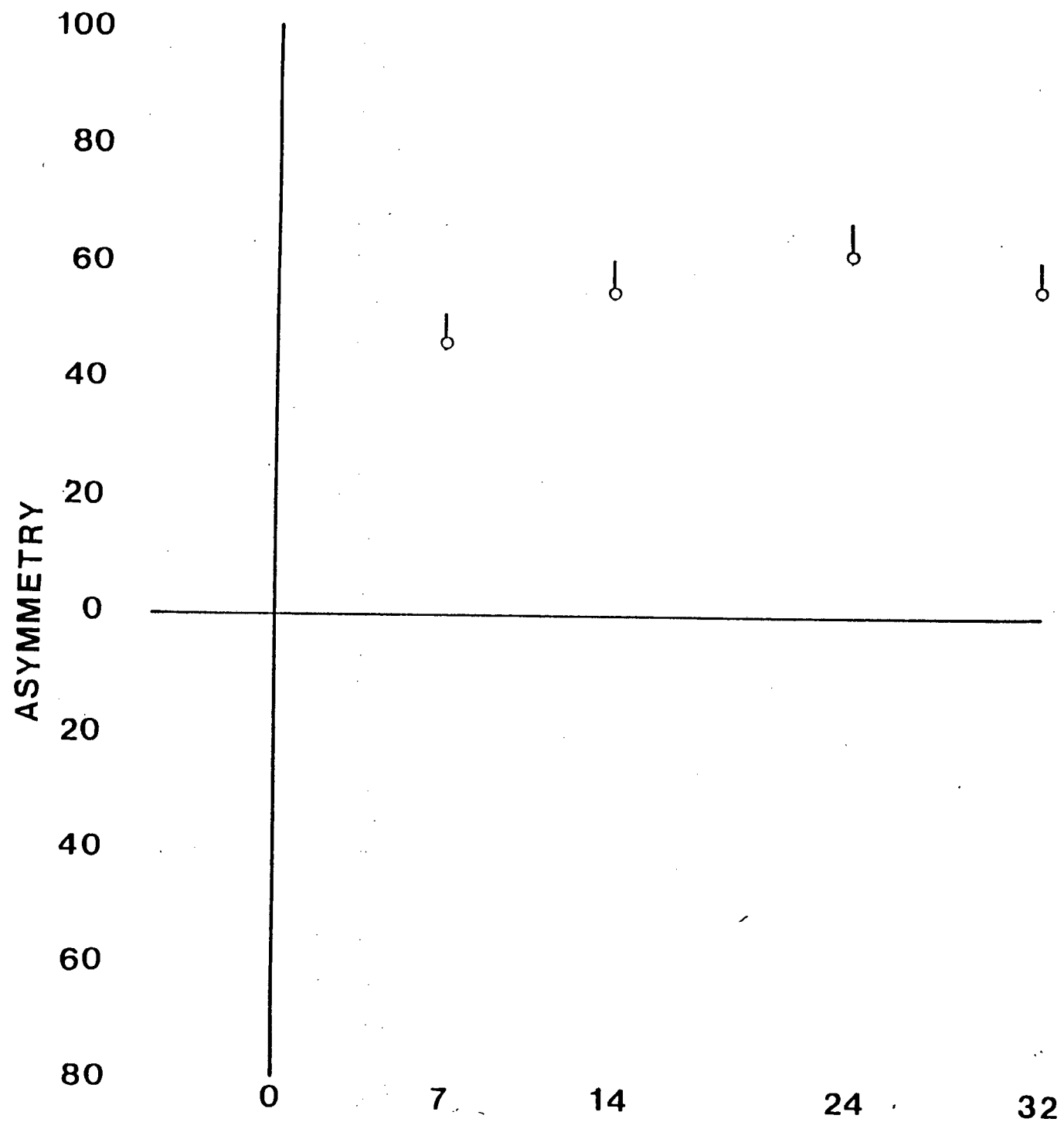


Table 4.20 Animals exhibiting recovery from amphetamine (1mg/Kg, i.p.) driven behaviour with restricted 6-OHDA lesion of the anteromedial SN. Data presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14, 24 and 32 are the days on which behaviour was tested post-lesion. * designates animal lesioned in the non-dominant hemisphere. n=14.

RAT	AP	LAT	D	S1	S2	7	14	24	32
94	3.0	0.8	8.5	3/7	0.75/0.75		203/226	246/253	111/155
95	3.0	1.0	8.6	2/2	0/0		301/302	196/205	50/58
113	2.4	1.0	9.2	4/10	-5/54	188/222	186/186	177/178	
139	2.4	1.6	9.6	-25/58	-132/142	268/273	254/263	230/234	
229	3.0	1.0	8.6	-5/60	0/6.5		374/390	161/175	254/266
254	3.6	1.0	7.4	-0.25/3	-20/35	364/366	168/171	121/141	98/102
*258	3.4	1.3	7.5	-20/35	-62/87	564/595	171/172	242/253	296/298
260	2.6	1.4	7.4	-56/78	-41/148	298/453	211/235	409/423	106/123
261	2.8	1.2	7.4	-1/17	-6/53	113/293	75/81	34/40	54/73
263	3.6	1.0	7.4	-45/135	40/94	373/381	284/288	284/290	269/269
270	3.2	1.0	7.4	-5/5	-7/11	121/139	87/89	165/170	65/66
271	3.2	1.0	7.4	5/65	-12/72	83/114	26/50	86/106	71/79
275	3.8	1.0	7.4	-2/12	-1/62	324/325	155/170	270/271	247/249
276	3.8	1.0	7.4	1/18	-30/35	185/218	29/63	9/18	93/112
Nett	3.12	1.09	7.9	-10.3	-19.6	262	180	187	143
+SEM	0.03	0.01	0.06	1.4	2.8	13	7.3	7.5	8
Total				36	57.2	307	192	197	154
+SEM				2.8	3.4	12.7	7.1	7.5	7.5

Nett rotations on day 32 significantly different from day 7, $p < 0.02$.

Total rotations on day 32 significantly different from day 7, $p < 0.02$.

Nett rotations on day 32 significantly different from S1, $p < 0.002$.

Nett rotations on day 7 significantly different from S1, $p < 0.002$.

Total rotations on day 32 significantly different from S1, $p < 0.002$.

Total rotations on day 7 significantly different from S1, $p < 0.002$. Mann Whitney U tests.

FIG. 4.18. Schematic representation of the rotational responses of animals with restricted anteromedial SN lesions to amphetamine (1mg/Kg, i.p.). Fig. of data presented in table 4.20, showing animals recovering from the lesion-induced ipsilateral rotation. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. The circles represent nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Data are mean and SEM. $n=14$. There is crossing of nett rotation direction on day 7 post lesion from contralateral to ipsilateral to the lesioned side, and a trend towards a decrease in nett ipsilateral rotations over the test period ($p<0.02$).

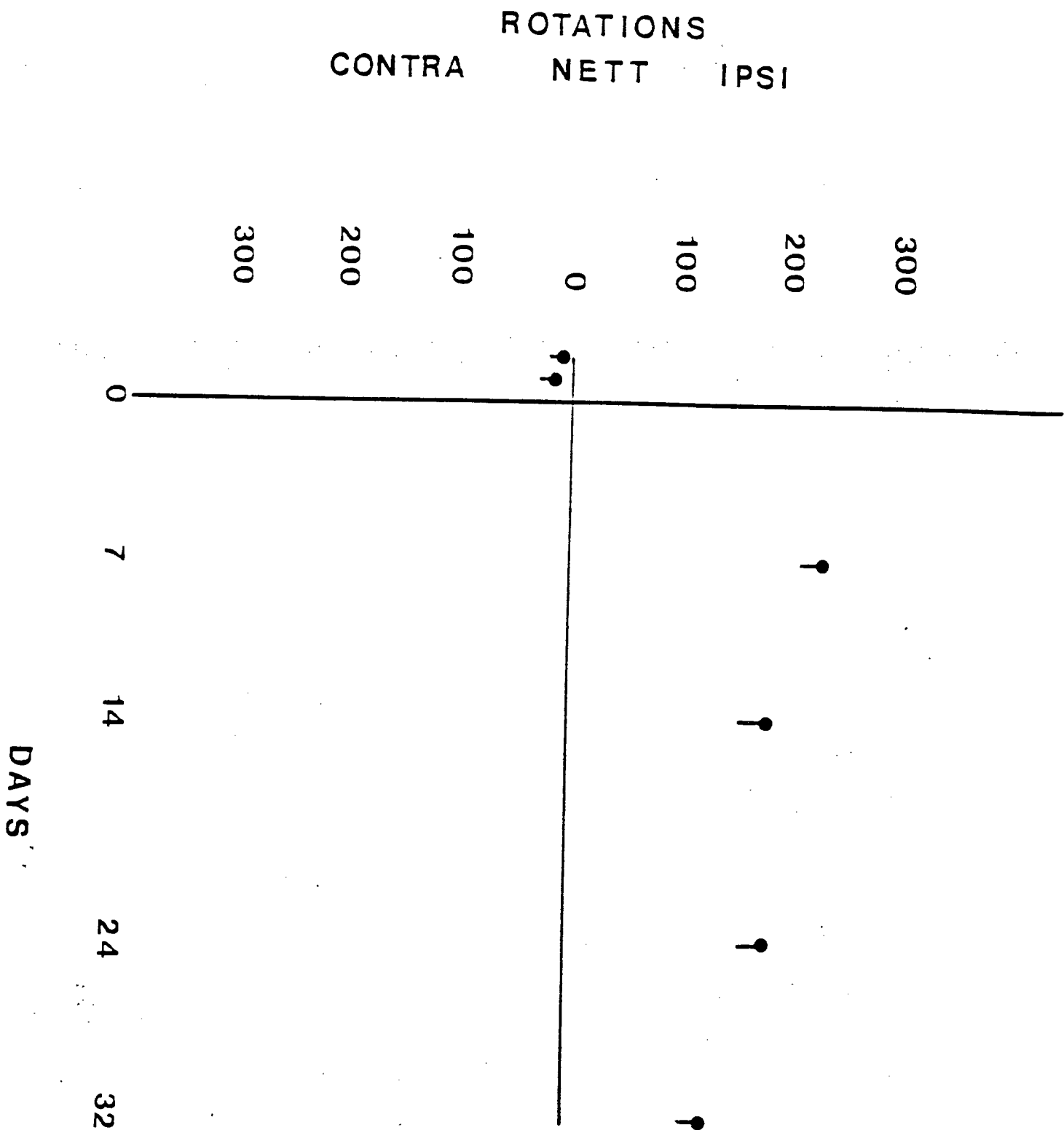


FIG. 4.19. Schematic representation of the rotational responses of animals with restricted anteromedial lesions of the SN to amphetamine (1mg/Kg). Fig. of data presented in table 4.20, showing animals recovering from the lesion-induced ipsilateral rotation. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. The triangles represent total rotations. Data are mean and SEM. $n=14$. The lesion increases the effect of the amphetamine, but there is a trend towards a decrease in total rotations over the test period ($p<0.02$).

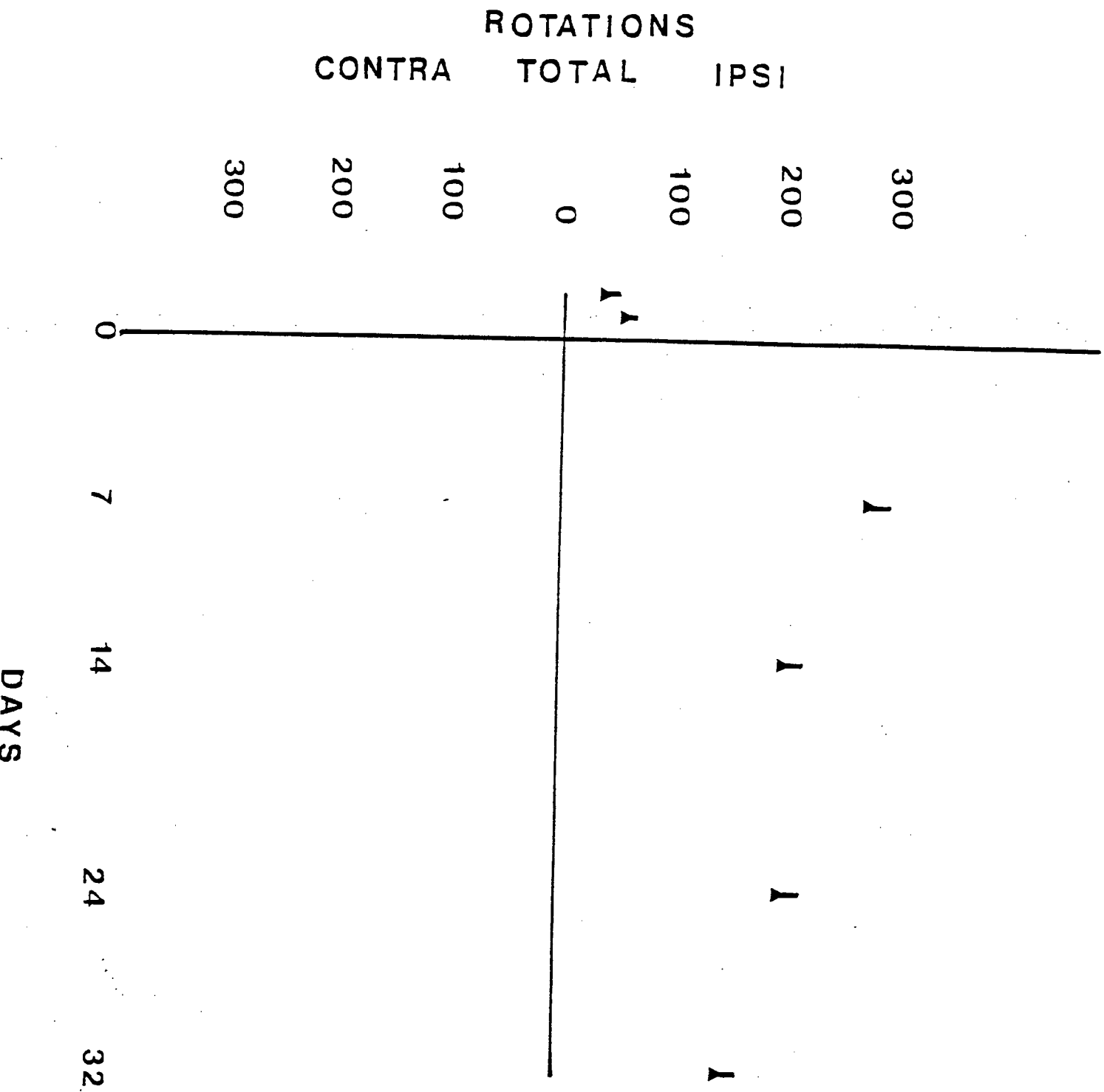


Table 4.21 Asymmetry index of animals exhibiting recovery as specified by a decrease in amphetamine (1mg/Kg, i.p.) driven nett ipsilateral rotations on the final test as compared to the first test. Amphetamine driven rotations expressed as $\text{Nett/Total} \times 100$. Positive values are ipsilateral to the lesioned side, negative contralateral. DAI is the change in the asymmetry index; +ve indicating an increase in asymmetry, -ve a decrease. n=14.

RAT	S1	S2	7	14	24	32	DAI
94	+43	+100		+90	+97	+72	-18
95	+100	0		+100	+96	+85	-15
113	+40	-10	+84	+100	+99	+15	-85
139	-42	-93	98	97	98		0
229	-9	0		+96	+92	+73	-23
254	-9	-57	+99	+98	+86	+96	-2
258	-57	-71	+95	+99	+96	+99	0
260	-72	-27	+66	+90	+97	+87	-3
261	-6	-12	+39	+93	+86	+74	-19
263	-33	43	+98	+99	+98	+100	+1
270	-100	-62	+87	+98	+97	+98	0
271	+7	-17	+73	+53	+81	+90	+37
275	-16	-2	+100	+91	+100	+99	+8
276	+6	-55	+85	+46	+48	+83	+37
Mean	+38.6	+39.2	+84	+89.3	+90.8	+88	+4.5
+--SEM	2.4	2.5	1.3	1.2	1.0	0.8	1.3

AI on day 32 significantly different from S1, $p < 0.002$.

AI on day 7 significantly different from S1, $p < 0.002$.

AI on day 32 not significantly different from day 7, $p > 0.1$

• Mann Whitney U tests.

FIG. 4.20. Asymmetry index ($AI = \text{Nett} / \text{Total} * 100$) of animals exhibiting recovery from anteromedial SN lesion as shown by decreased amphetamine (1mg/Kg, i.p.) driven nett rotations. Fig. of data presented in table 4.21. Data are mean and SEM. n=14. The AI for amphetamine-driven rotations is higher than for spontaneous rotations. There is no change in the AI over the test period.

ASYMMETRY

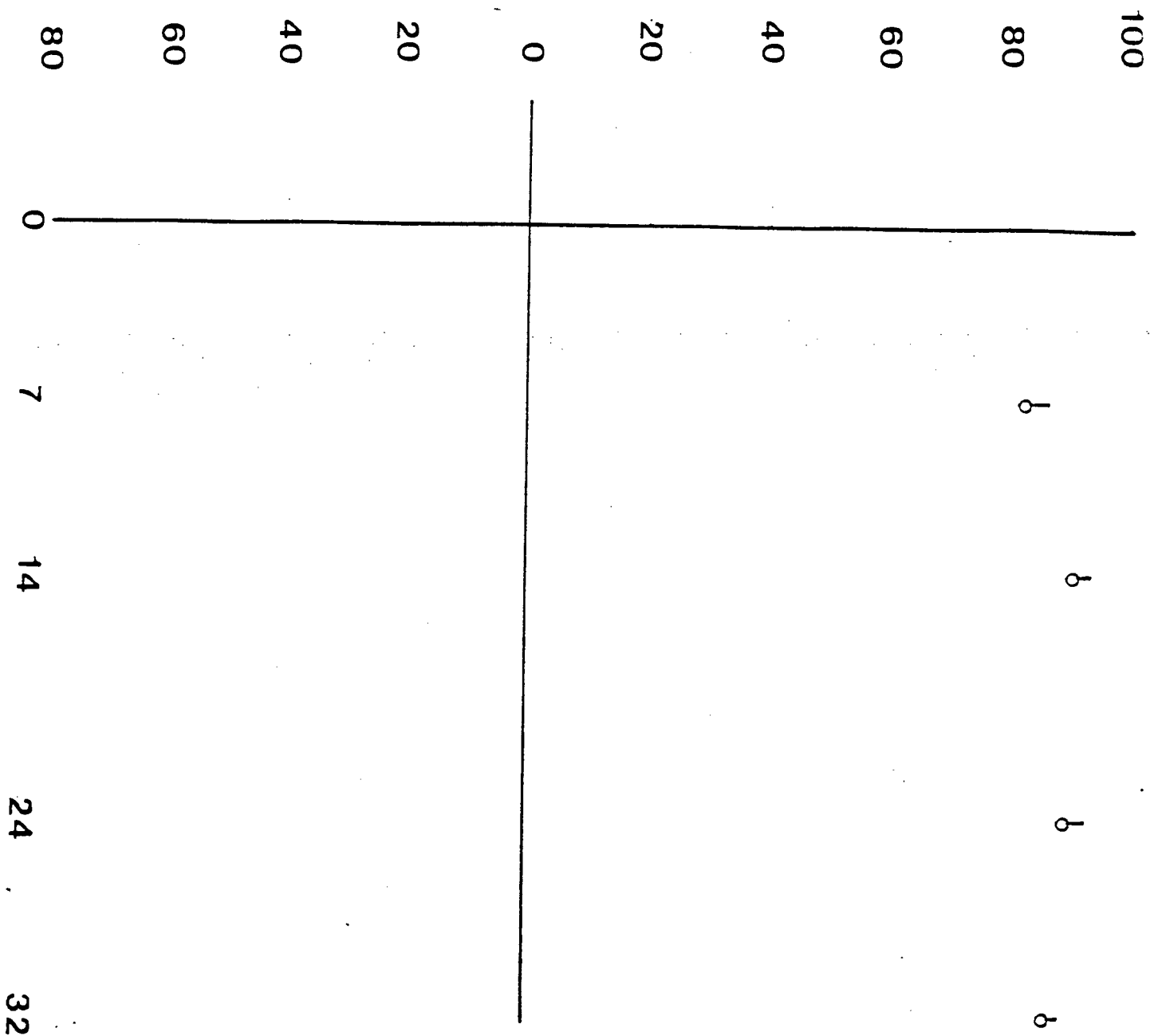


Table 4.22 Animals not exhibiting recovery from spontaneous rotation with restricted 6-OHDA lesion of the anteromedial SN lesion. Data presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14, 24 and 32 are the days on which behaviour was tested post-lesion. * indicates lesion of the non-dominant hemisphere. n=14.

RAT	AP	LAT	D	S1	S2	7	14	24	32
98	3.0	1.0	8.6	4/4	1/6.5		16/17.5	12.5/32	16/18
108	2.4	0.8	9.1	-4/9	-0.5/1.5	-3/4	26/26	7.5/7.5	
113	2.4	1.0	9.2	-4.5/16.5	-8.5/22.5	6/6	4.5/8.5	15/15.5	
137	3.0	1.3	9.6	-5.5/24	6/31	4/10	8.5/12	7.5/9	
139	2.4	1.4	9.6	-6/18	-7.5/8.5	13/26	11/24	20/31.5	
229	3.0	1.0	9.6	1.5/52	-4.5/6.5		0/11	7.5/22	7.5/11
*262	3.2	1.0	7.6	1/16	4/8	2.5/6	9/13	1/5.5	6/11
263	3.6	1.0	7.4	-11/47	-1/37	20/24	14/23	31/49	41/46
264	2.8	1.0	7.6	1/41	-3/39	2/6	5.5/12	3/21	9/25
265	3.2	1.2	7.6	2/9.5	-0.5/14	9/14.5	4/14.5	10/19	12/24
270	3.2	1.0	7.4	-10/13	-12/12	5/12	10/13	8/11	18/21
271	3.2	1.0	7.4	4.5/8.5	-3/20	10/13	16/34	7/20	15/19
275	3.8	1.0	7.4	6/68	-4/40	34/45	26/32	9/9.5	42/44
277	3.6	0.5	7.2	-4/38	0/17	27/76	40/68	20/33	31/57
Nett	3.06	1.02	8.16	-1.8	-2.4	10.8	13.6	11.4	19.8
+SEM	0.03	0.02	0.06	0.4	0.3	0.9	0.8	0.6	1.3
Total				26	18.8	20.2	22	20.4	27.6
+SEM				1.4	0.9	1.8	1.1	0.9	1.6

Nett rotations on day 32 not significantly different from day 7, $p > 0.01$.

Total rotations on day 32 not significantly different from S1, $p > 0.1$.

Nett rotations on day 32 significantly different from S1, $p < 0.002$.

Nett rotations on day 7 significantly different from S1, $p < 0.002$.

Total rotations on day 32 not significantly different from S1, $p > 0.1$.

Total rotations on day 7 not significantly different from S1, $p > 0.1$.

Mann Whitney U tests.

FIG. 4.21. Schematic representation of the spontaneous rotational responses of animals with anteromedial SN lesions. Fig. of data presented in table 4.22, showing animals not recovering from the lesion-induced ipsilateral rotation. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles represent nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Data are mean and SEM. n=14. There is crossing of nett rotation direction on day 7 from contralateral to ipsilateral to the lesioned side. There is no trend towards a decrease in rotations over the test period.

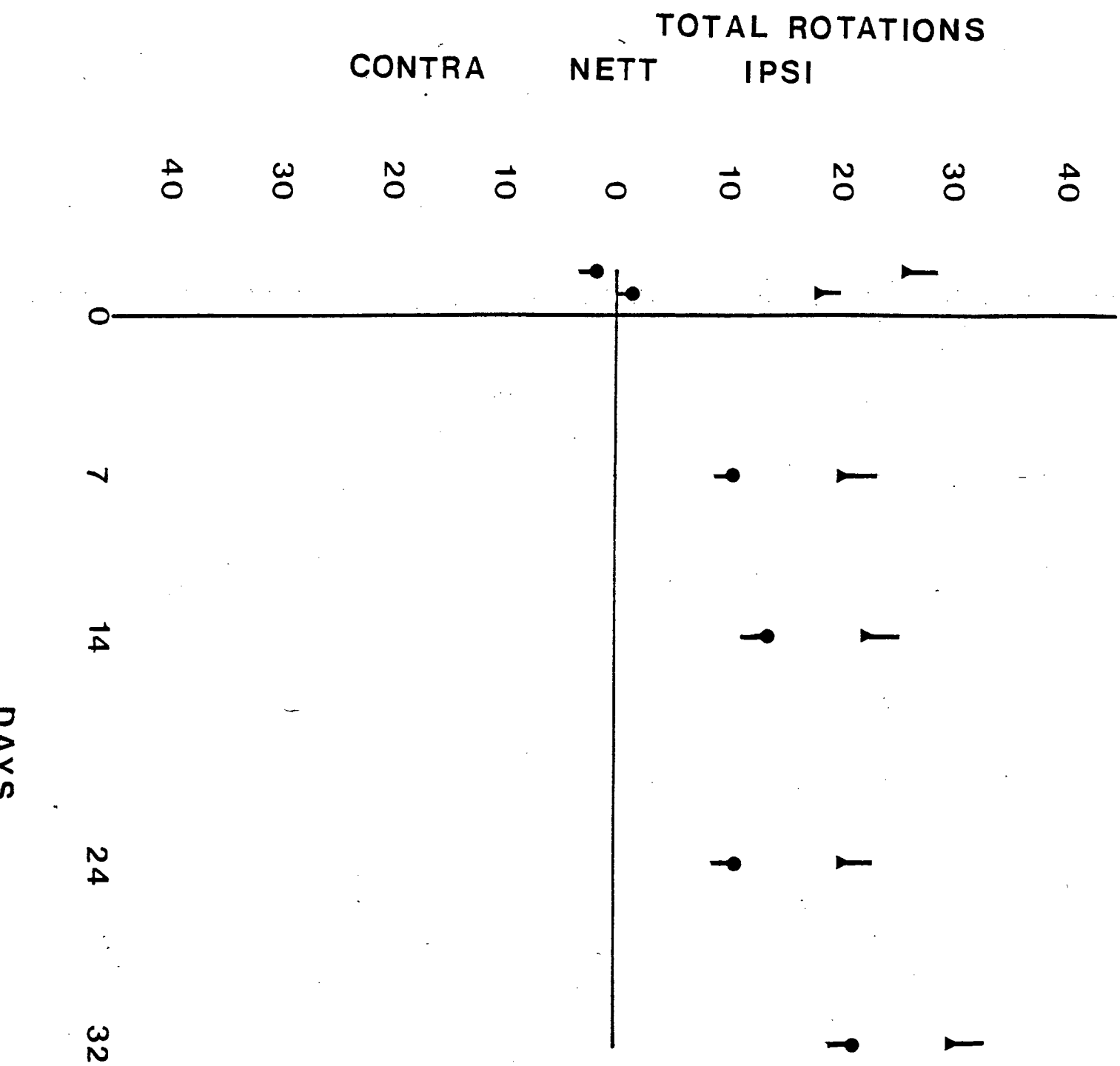


Table 4.23 Asymmetry index of animals not exhibiting recovery as specified by a decrease in spontaneous nett ipsilateral rotations on the final test as compared with the first test. spontaneous rotations expressed as $\text{Nett/Total} \times 100$. Positive values are ipsilateral to the lesioned side, negative contralateral. DA is the change in the asymmetry index; +ve indicating an increase in asymmetry, -ve a decrease. $n=14$.

RAT	S1	S2	7	14	24	32	DA
98	-100	+14		+89	+39	+90	+1
108	-47	-33	+78	+100	+100		+22
113	-28	-39	+100	+56	+94		-6
137	-22	-19	+43	+69	+80		+37
139	-33	-89	+53	+49	+65		+12
229	+3	-71		0	-35	-68	+68
262	+6	+52	+42	+70	+18	+52	-18
263	-24	-2	+81	+61	+63	+90	+29
264	+2	-8	+33	+46	+14	+37	-9
265	+21	-4	+62	+28	+52	+48	+20
270	-80	+100	+39	+80	+72	+88	+8
271	+5	-14	+77	+46	+33	+81	+35
275	+9	-10	+76	+83	+95	+97	+14
277	-11	0	+35	+58	+60	+55	-3
Mean	+27.9	+32.5	+59.9	+59.6	+58	+70.6	+15
+--SEM	2.1	2.4	1.6	1.8	2.0	1.5	1.6

AI on day 32 significantly different from S1, $p < 0.002$.

AI on day 7 significantly different from S1, $p < 0.002$.

AI on day 32 not significantly different from day 7, $p > 0.1$.

. Mann Whitney U tests.

Table 4.24. Animals not exhibiting recovery from amphetamine (1mg/kg, i.p.) driven behaviour with restricted 6-OHDA lesion of the anteromedial SN. Data presented as Nett/Total rotations per hour. -ve indicates rotation contralateral to the lesioned side. S represents the pre-lesion screens, while 7, 14, 24, and 32 are the days on which behaviour was tested post-lesion. * indicates animal lesioned in the non-dominant hemisphere. n=18.

RAT	AP	LAT	D	S1	S2	7	14	24	32
98	3.0	1.0	8.6	-2/18	-34/46		225/232	282/285	414/420
100	3.0	0.8	9.1	-53/54	-5/16		70/72	37/38	176/200
103	2.4	1.3	9.5	2/22	-2/20	36/45	105/121	61/74	
108	2.4	0.8	9.1	7/40	-24/106	146/147	114/117	152/156	
137	3.0	1.3	9.6	-2/40	-2.32	5/16	67/71	73/81	
225	3.8	1.6	8.6	-5.7	-2/5		87/99	232/240	167/176
228	3.0	1.2	8.8	0.5/43	-30/51		133/143	334/341	291/296
230	3.0	1.3	8.6	-6/60	-0.25/28		216/236	296/326	254/266
255	3.2	1.0	7.4	-55/87	-64/99	178/383	163/183	271/301	188/201
256	3.0	1.0	7.4	-0.5/54	-32/106	228/247	104/118	166/177	291/299
257	3.4	0.5	7.4	2/36	-26/106	77/117	62/120	144/264	216/250
*259	3.2	1.2	7.6	63/191	74/209	231/304	303/322	501/507	415/431
*262	3.2	1.0	7.6	74/75	53/55	136/163	217/227	210/222	158/165
264	2.8	1.0	7.6	-6.5/10	56/163	41/153	227/237	129/185	158/194
265	3.2	1.2	7.6	-2.5/30.5	-15/55	287/299	97/181	272/278	391/393
272	3.4	0.8	7.2	9/35	-6/62	121/130	278/287	273/289	327/341
273	3.0	1.0	8.1	-63/174	-149/209	-9/73	-13/102	121/143	333/384
277	3.6	0.2	7.2	0/35	-4/46	32/57	52/71	87/115	147/165
Mean	3.08	1.01	8.16	-2.1	-11.8	116	139.3	202.3	261
+SEM	0.02	0.02	0.05	1.9	2.7	7.3	4.8	6.5	6.5
Total				56.2	78.5	164	163.3	223.4	278
+SEM				2.8	3.45	8.7	4.3	6.4	6.4

Nett rotations on day 32 significantly different from day 7, $p < 0.002$.

Total rotations on day 32 significantly different from day 7, $p < 0.02$.

Nett rotations on day 32 significantly different from S1, $p < 0.002$.

Nett rotations on day 7 significantly different from S1, $p < 0.002$.

Total rotations on day 32 significantly different from S1, $p < 0.002$.

Total rotations on day 7 significantly different from S1, $p = 0.002$.

Mann Whitney U tests.

FIG. 4.22. Schematic representation of the rotational responses of animals with restricted anteromedial SN lesions to amphetamine (1mg/Kg, i.p.). Fig. of data presented in table 4.24, showing animals not recovering from the lesion. Lesion is at day 0 on the x-axis. Rotational behaviour was measured on days 7, 14, 24, and 32 post lesion. In addition, screening of naive animals was carried out on two consecutive days prior to the lesion. Triangles represent total rotations, circles nett rotations. Nett ipsilateral rotations are represented above the x-axis, nett contralateral rotations below. Data are mean and SEM. n=18. There is crossing of nett rotation direction on day 7 from contralateral to ipsilateral to the lesioned side. There is no apparent recovery over the test period.

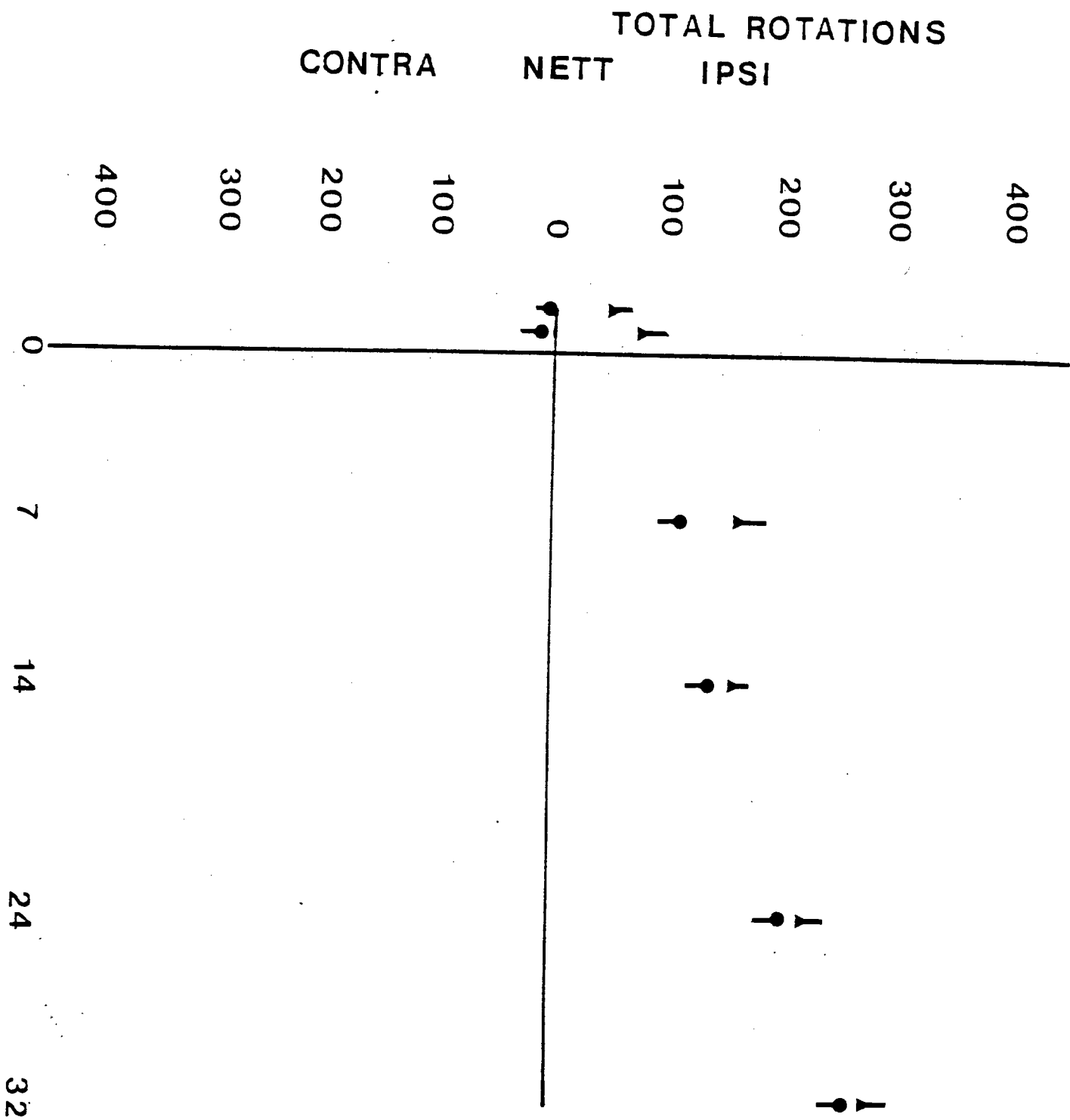


Table 4.25 Table of an animal not exhibiting recovery as specified by a decrease in nett amphetamine (1mg/Kg, i.p.) driven ipsilateral rotations on the final test as compared to the first test. Amphetamine driven rotations expressed as Nett/Total*100. Positive values are ipsilateral to the lesioned side, negative contralateral. DA is the change in the asymmetry index; +ve indicating an increase in asymmetry, -ve a decrease. n=18.

RAT	S1	S2	7	14	24	32	DA
98	-10	-75		+97	+99	+99	+2
100	-99	-32		+98	+99	+98	-10
103	+10	-11	+79	+86	+82		+3
108	+17	-22	+99	+98	+97		-2
137	-4	-6	+33	+94	+90		+57
225	-72	-47		+88	+97	+95	+7
228	+1	-60		+93	+98	+98	+5
230	-9	-1		+92	+91	+95	+3
255	-63	-65	+46	+89	+90	+94	+48
256	+1	-30	+92	+89	+94	+97	+5
257	+6	-25	+66	+52	+53	+86	+20
259	+33	135	+76	+94	+99	+96	+20
262	+99	+96	+83	+96	+95	+95	+12
264	-65	+34	+27	+96	+70	+82	+55
265	-8	-27	196	+54	+98	+99	+3
272	+25	+10	+93	+97	+94	196	+3
273	-36	-71	-12	-13	+85	+87	+99
277	0	-12	+56	+73	+76	+89	+33
Mean	+31	+36.6	+66	+83.3	+89.3	+93	+20.1
+-SEM	1.8	1.5	1.6	1.2	0.7	0.3	1.5

AI on day 32 significantly different from S1, $p < 0.002$.

AI on day 32 significantly different from S2, $p < 0.002$

AI on day 32 significantly different from day 7. $p < 0.02$

Mann Whitney U tests.

FIG. 4.23. Locations of restricted 6-OHDA lesions of the anteromedial SN. #94 to #230 are schematic diagrams based on the atlas of Pelligrino et al. (1979). The grey areas show the extent of the lesion. Involutated cells were found within this area. The black areas show regions of non-specific damage in the form of severe gliosis or vacuolation. The lesions were generally of the anteromedial SN and VTN. There was severe gliosis in some animals only at the cannula release site. The SNR and PC were unaffected. The HRP investigation of #254 to #277 revealed greatly diminished cell counts when lesioned animals are compared to controls. The results of this study are illustrated with camera lucida drawings of sections showing the lesion site and distribution of HRP filled cells. The AP value shown at top right is the nearest equivalent Pelligrino et al. (1979) section. HRP filled cells are represented by filled circles. The lesion is represented by a halo of stippling, often with a vertical line extending into it, designating the cannula track. The focus of the HRP deposition site is illustrated above each description. CP posterior commissure, DTV ventral tegmental decussation, HP habenulo-interpeduncular tract, LM medial lemniscus, MFB medial forebrain bundle, SN substantia nigra, VTN ventral tegmental nucleus of Tsai.

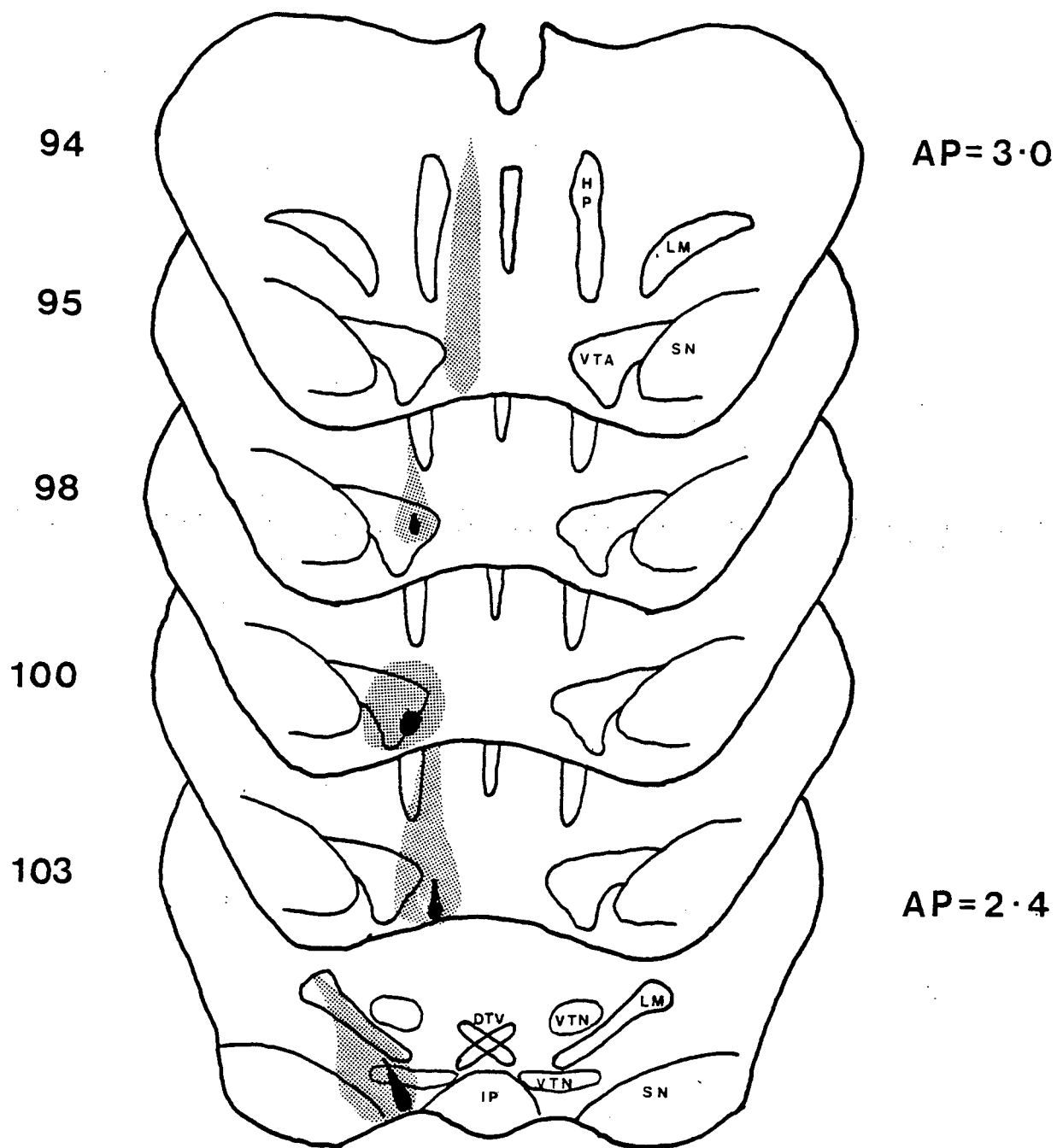


Fig. 4.23 continued.

108

AP=2.4

113

137

139

225

AP=3.8

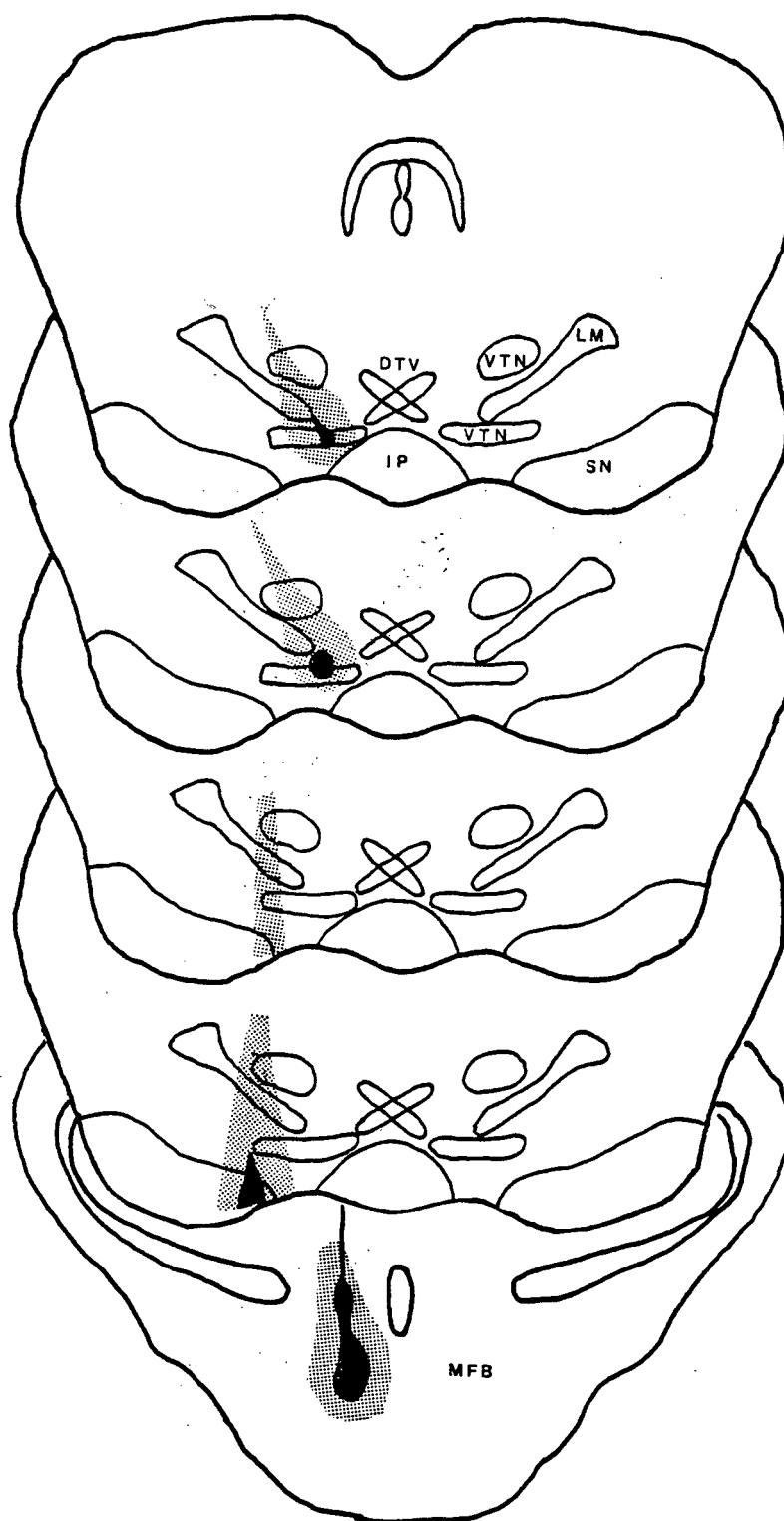


Fig. 4.23 continued.

228

AP=3.0

229

230

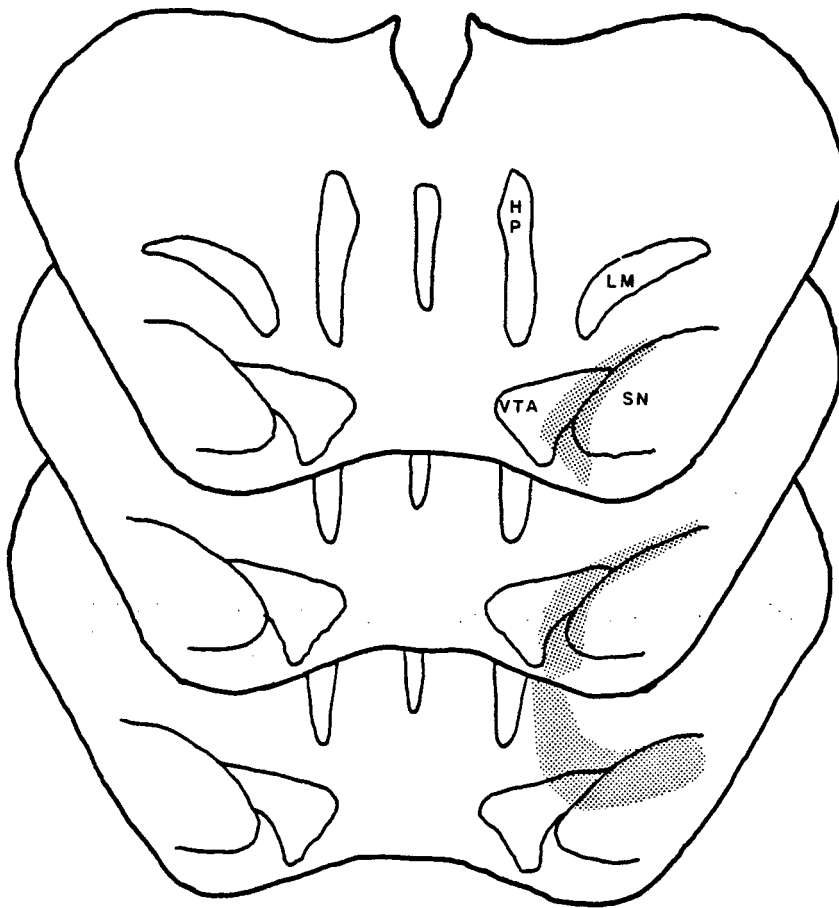
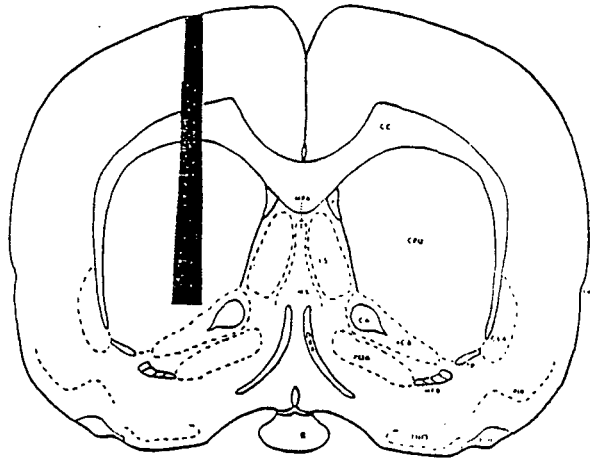
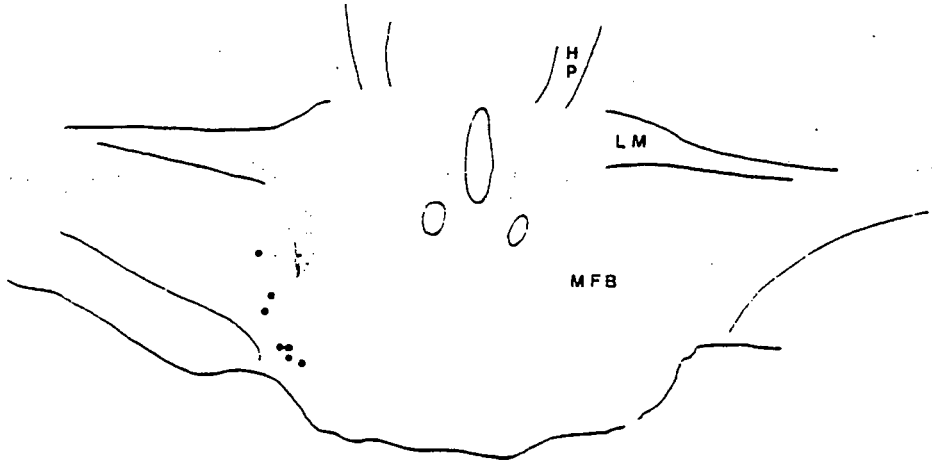


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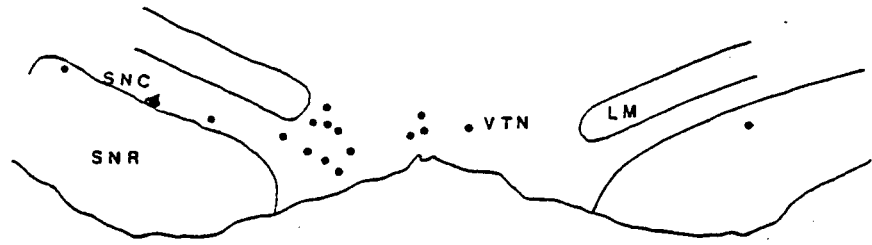
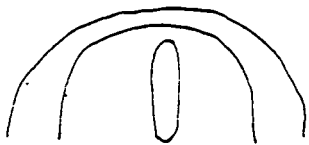


254

3·6



3·4



2·6

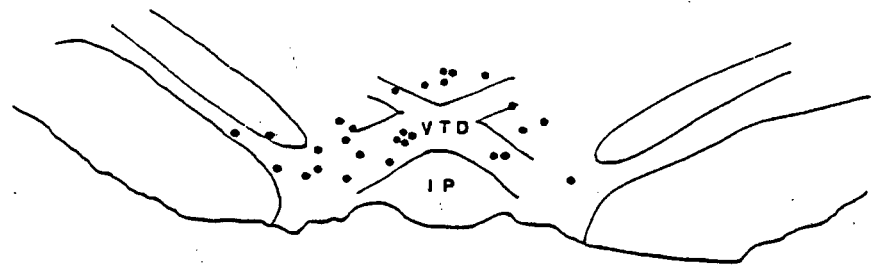


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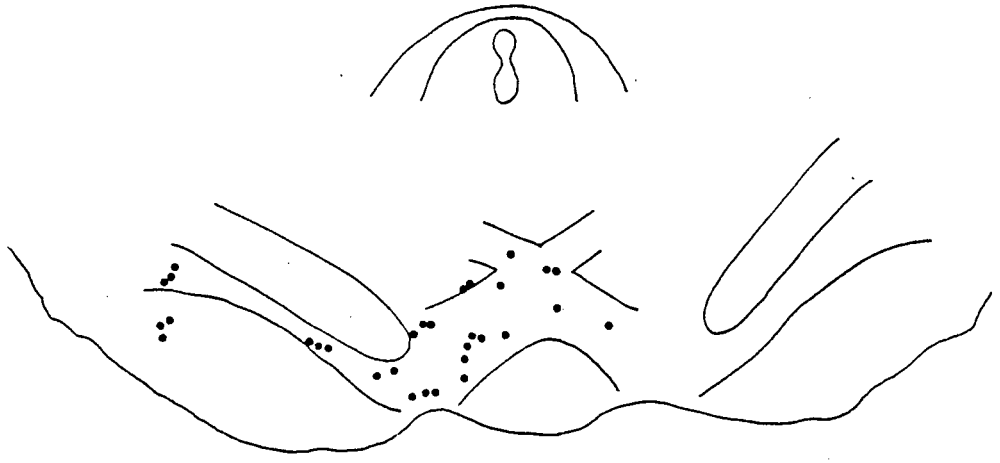
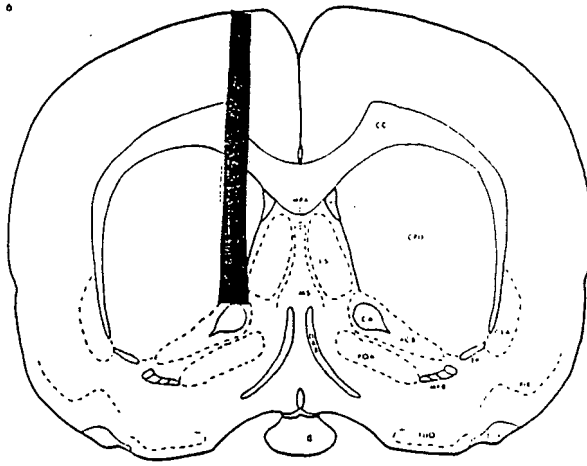
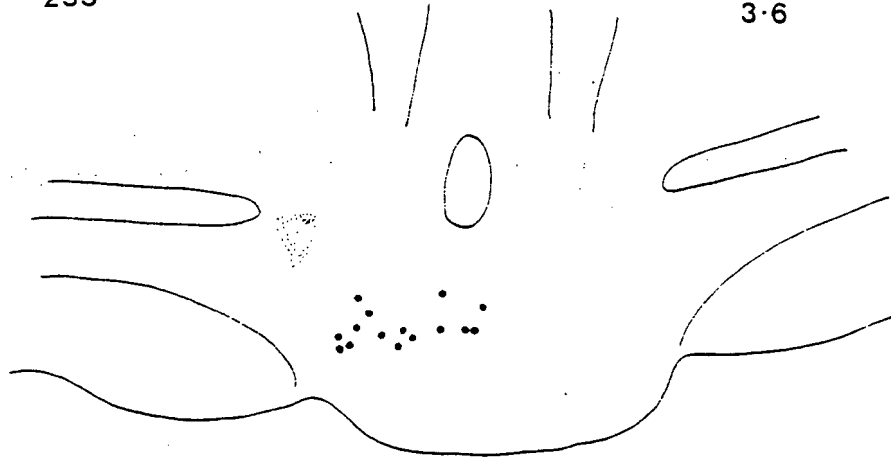


Fig. 4.23 continued.



255

3.6



2.4

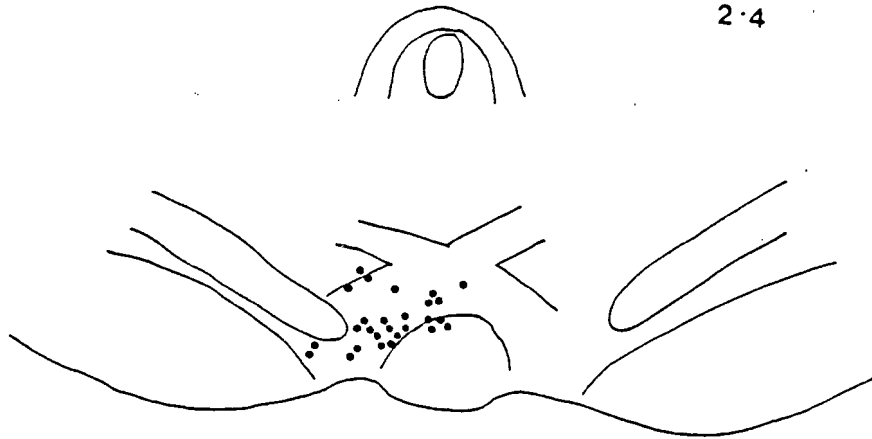
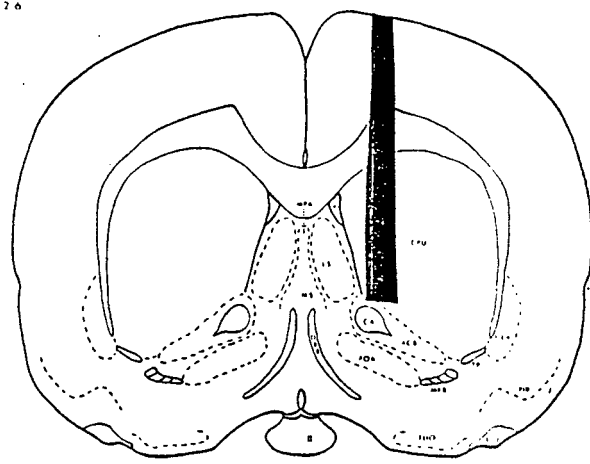
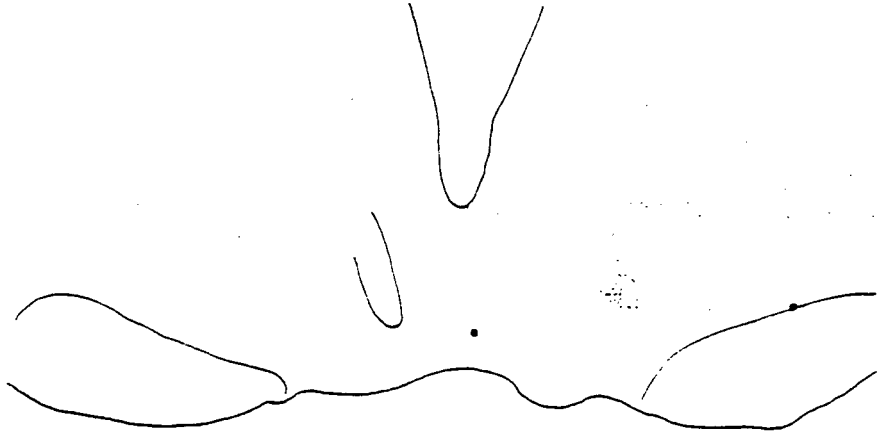


Fig. 4.23 continued.



256

3·0



2·4

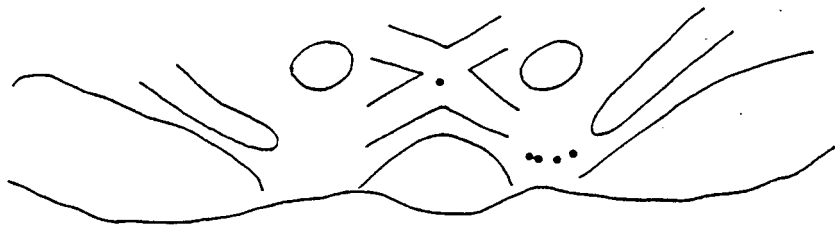
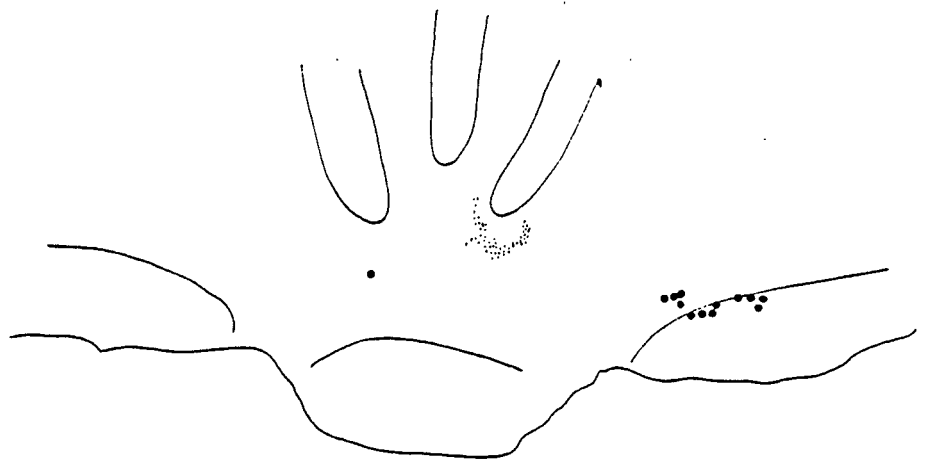
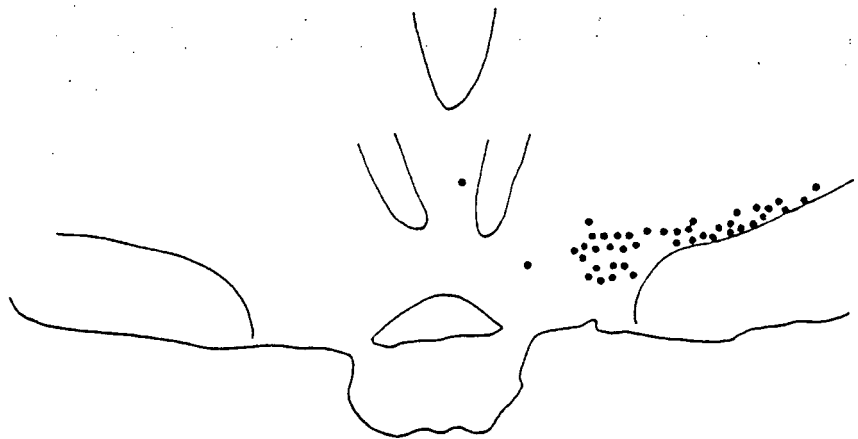


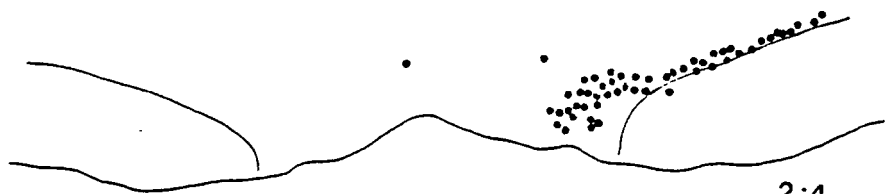
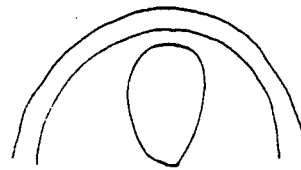
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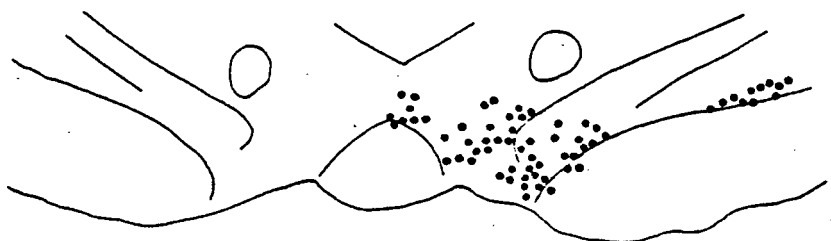
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2.8

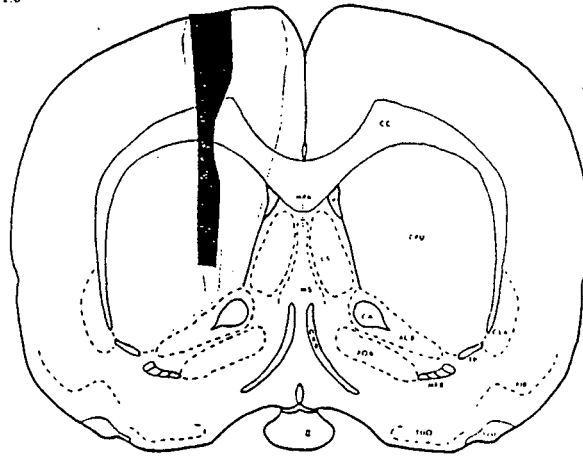


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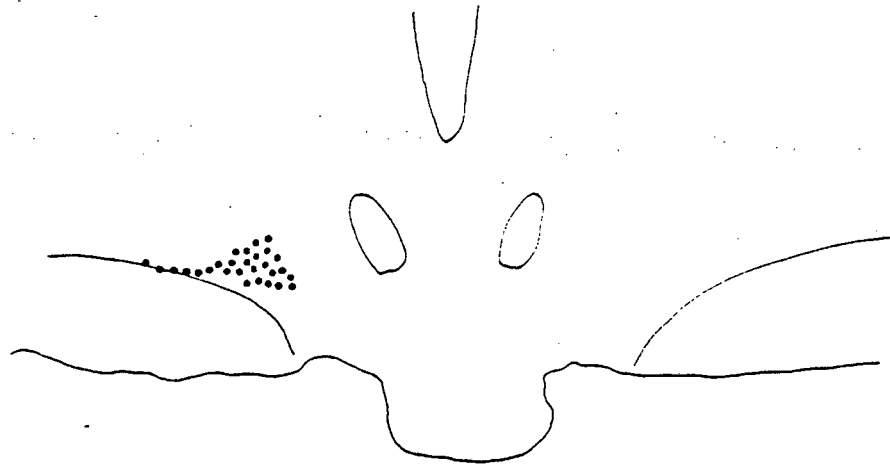
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Fig. 4.23 continued.



258

3.2



2.4

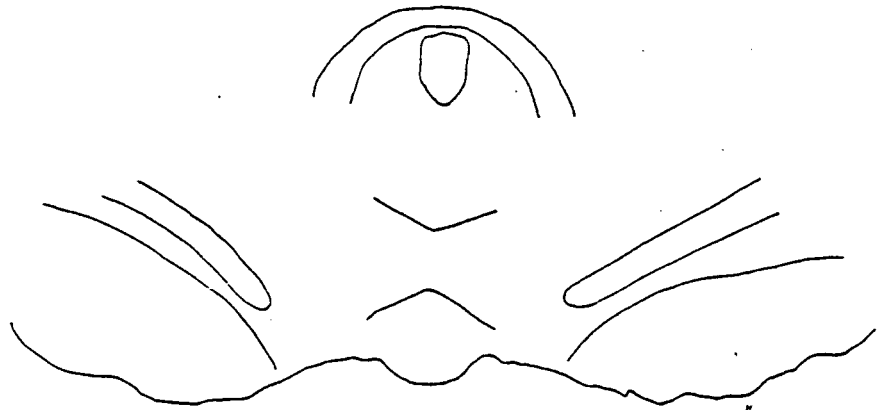
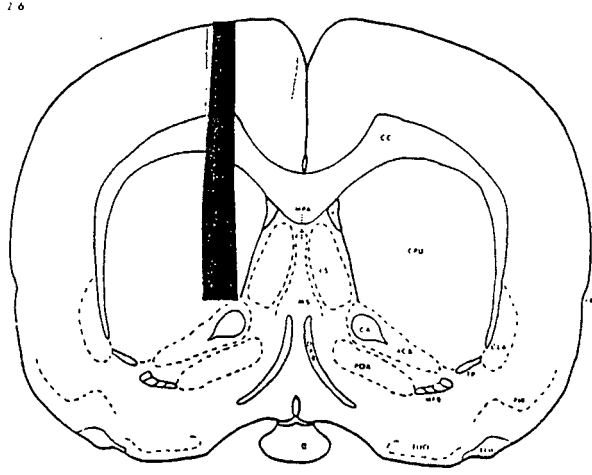


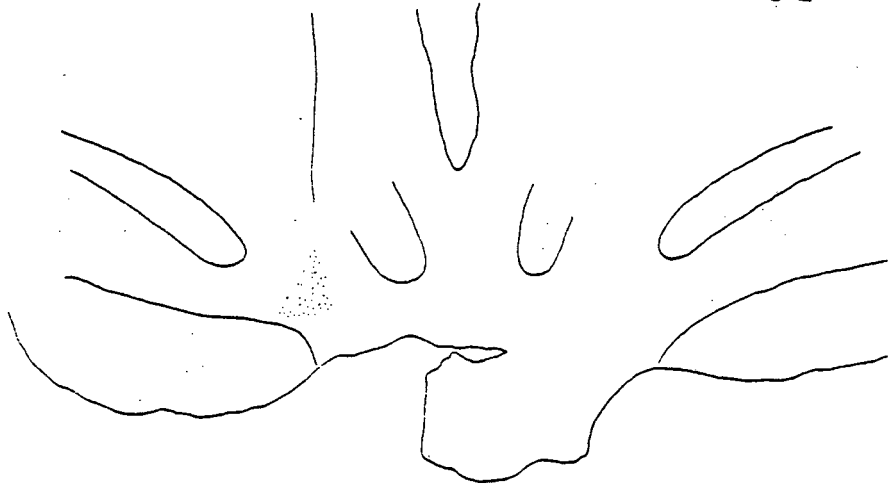
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Fig. 4.23 continued.

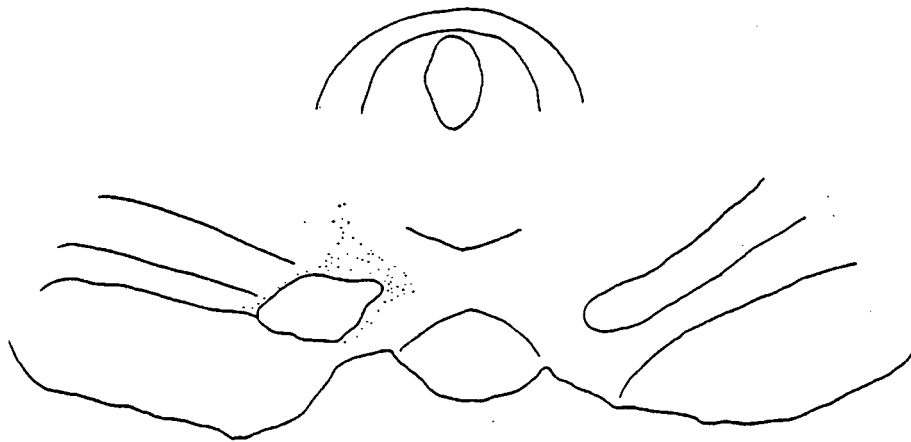


260

3.2



2.4



2.0

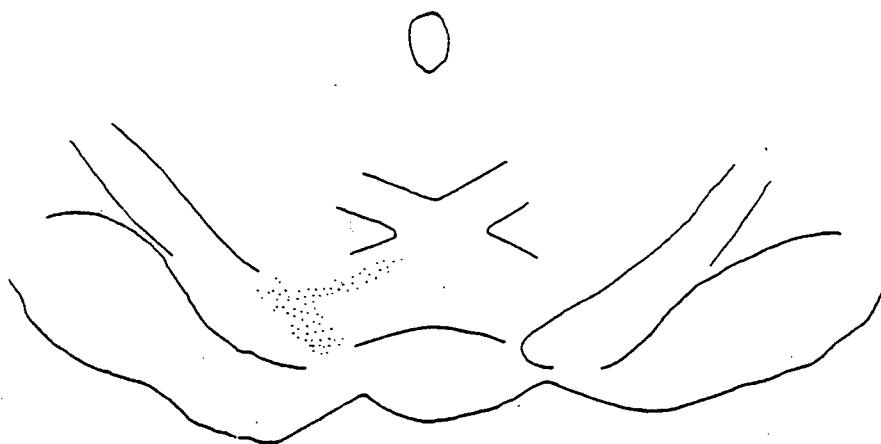
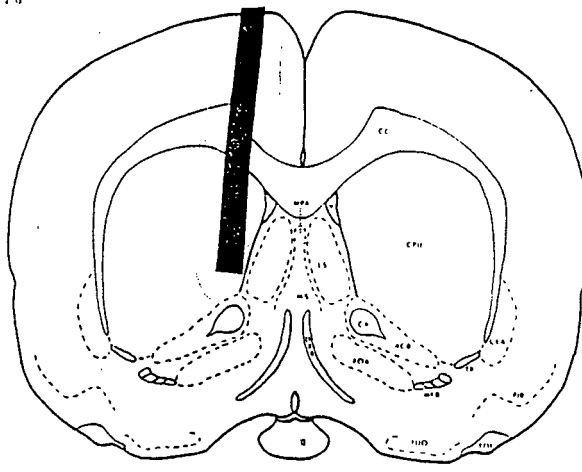
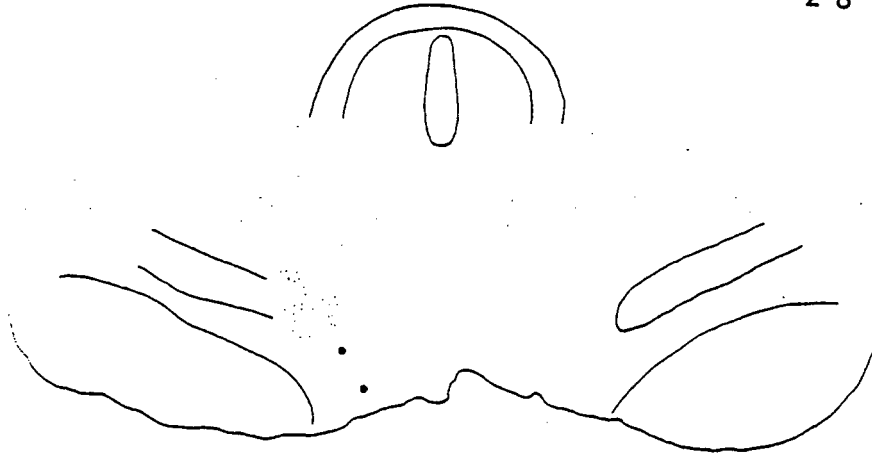


Fig. 4.23 continued.



261

2.8



2.4

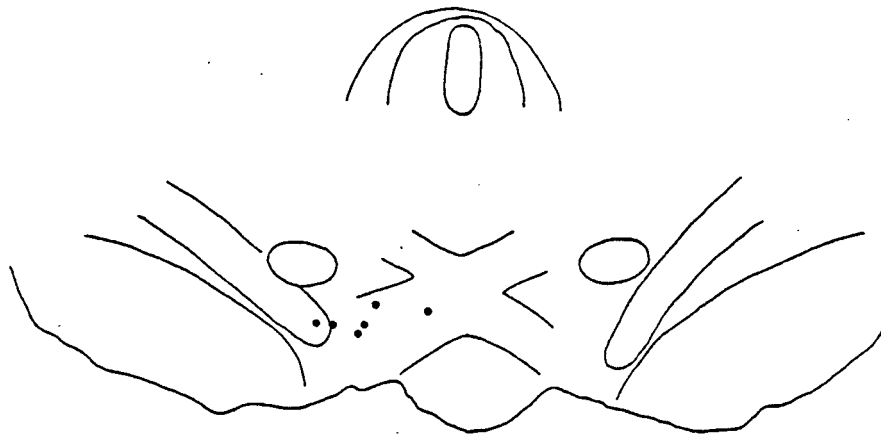
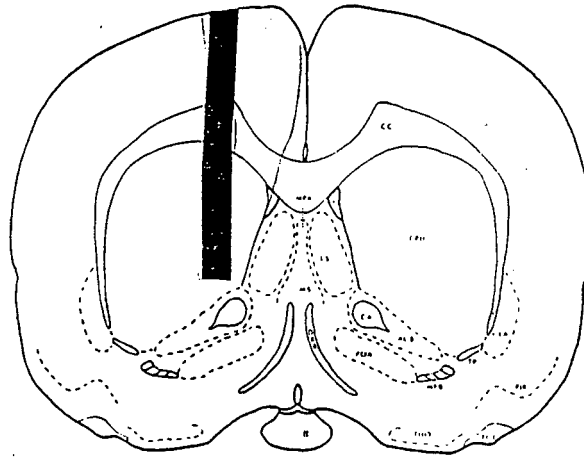
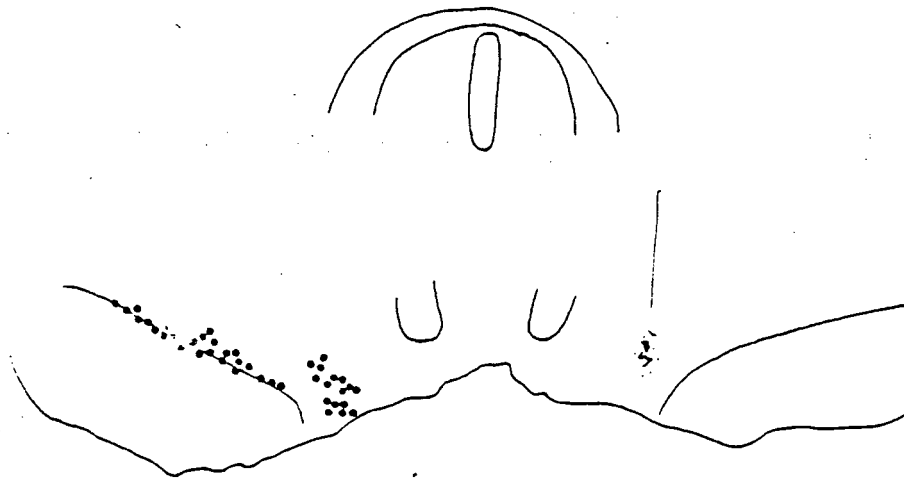


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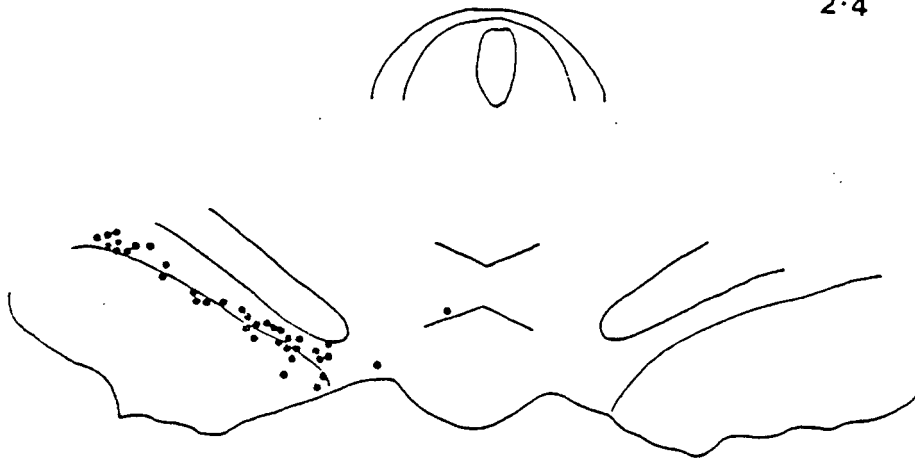


262

3.0



2.4



2.2

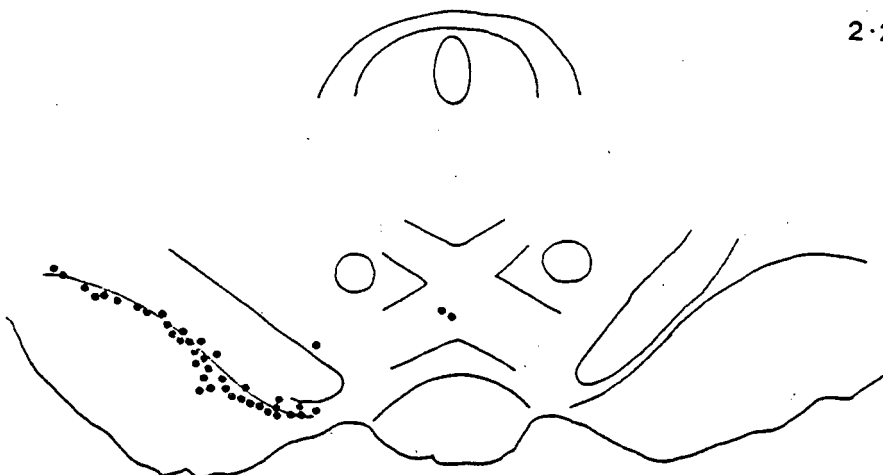
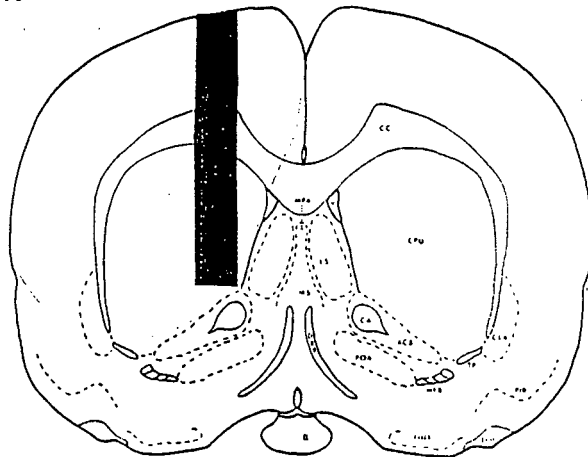
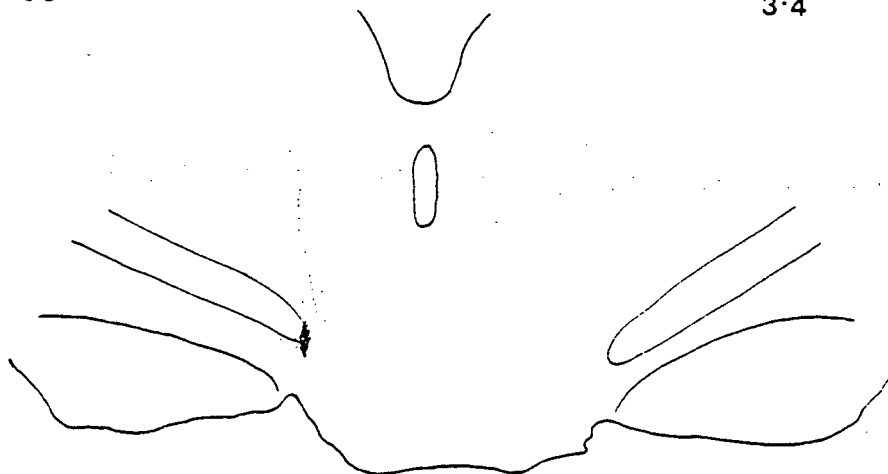


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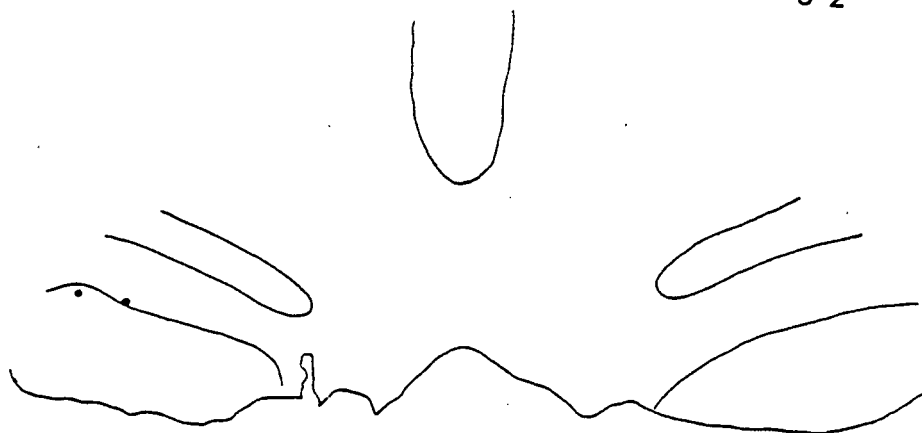


263

3·4



3·2



2·4

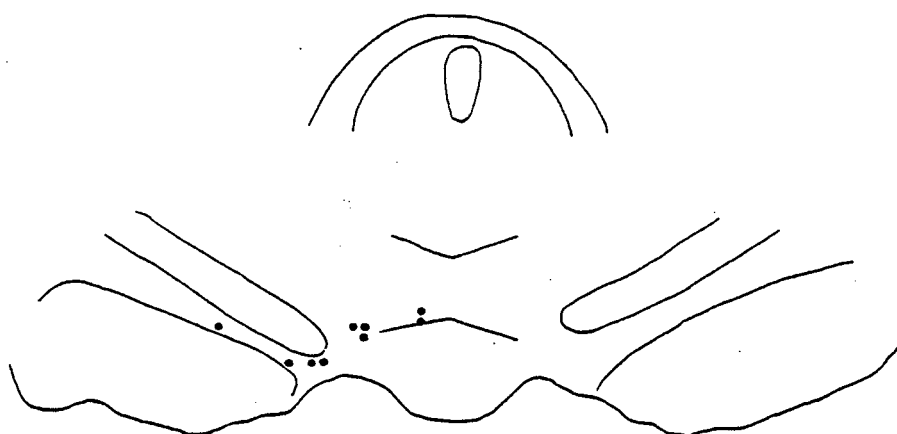
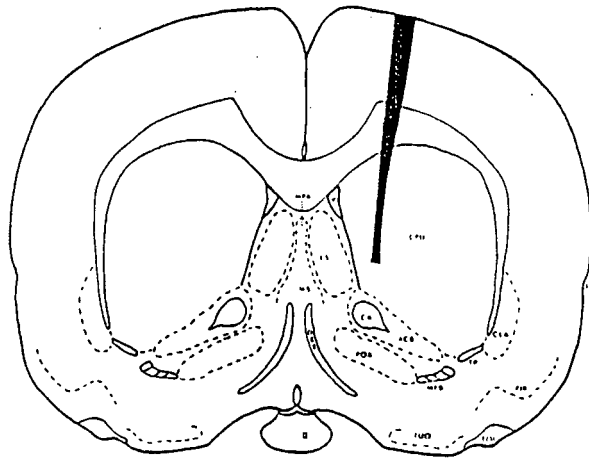
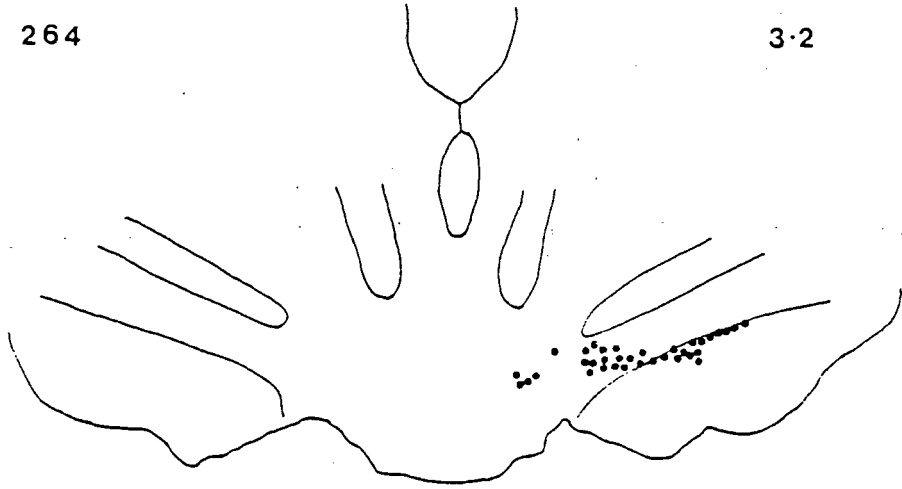


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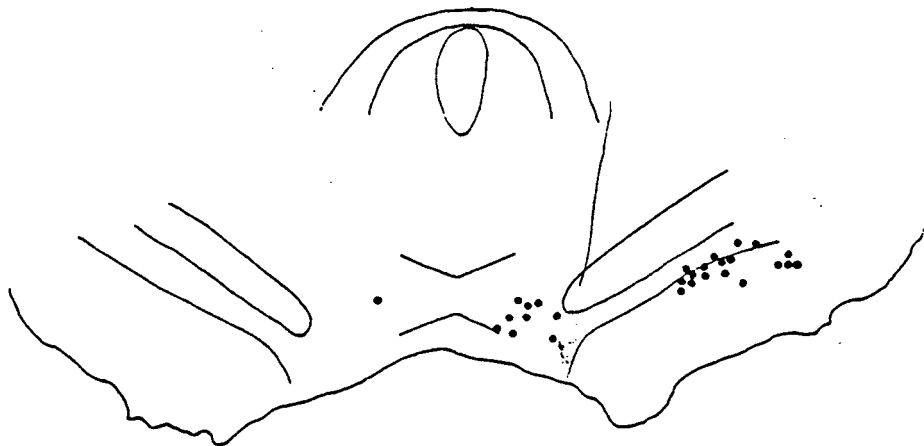


264

3.2



2.8



2.4

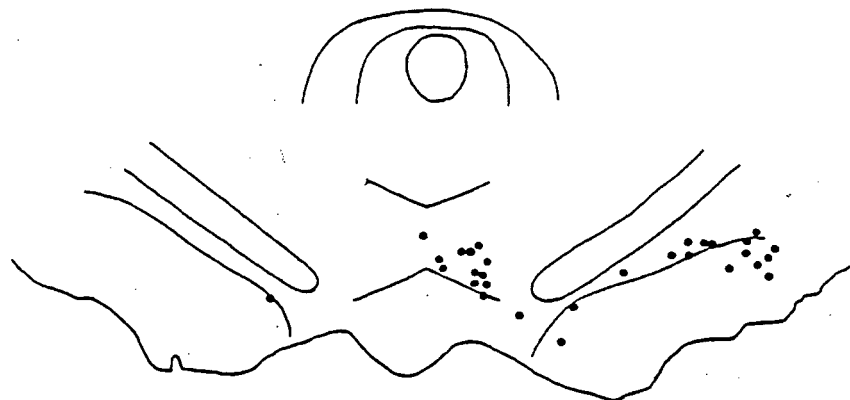
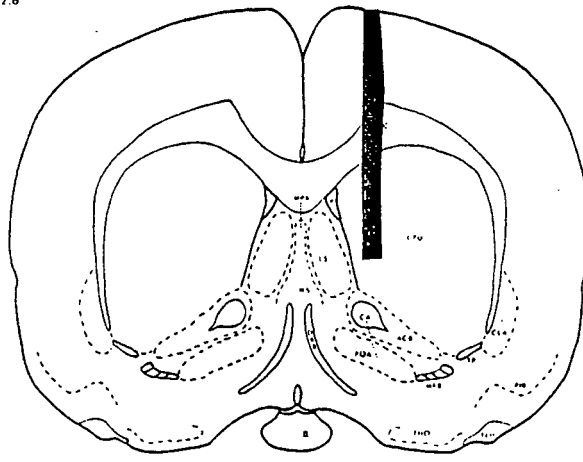
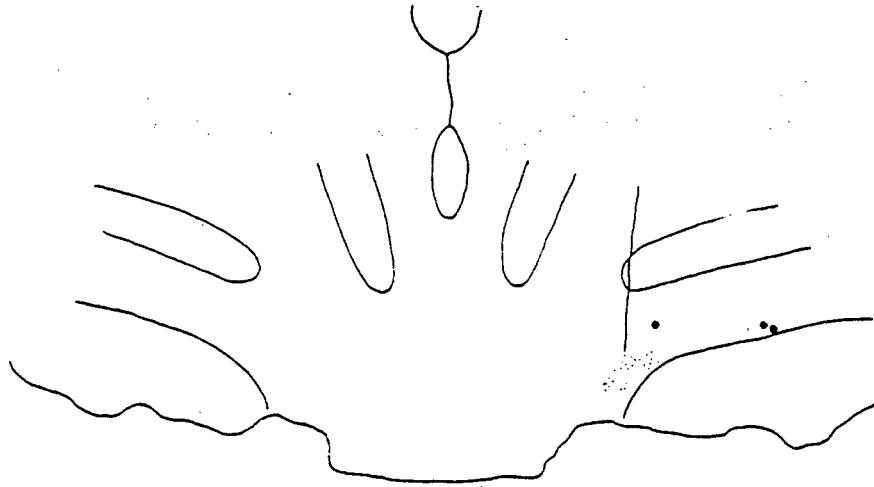


Fig. 4.23 continued.



265

3.2



2.4

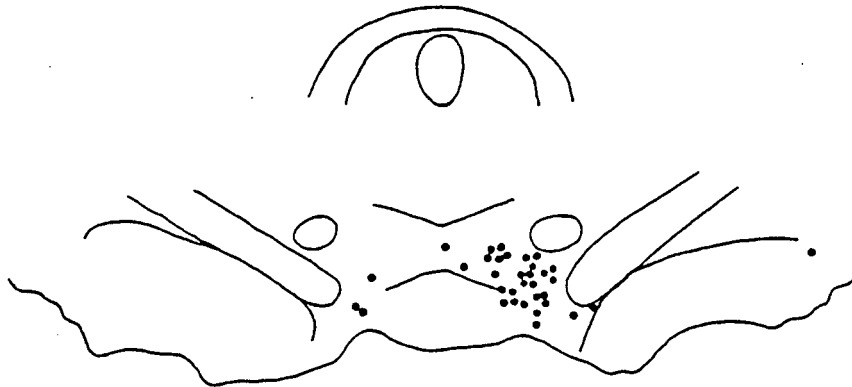
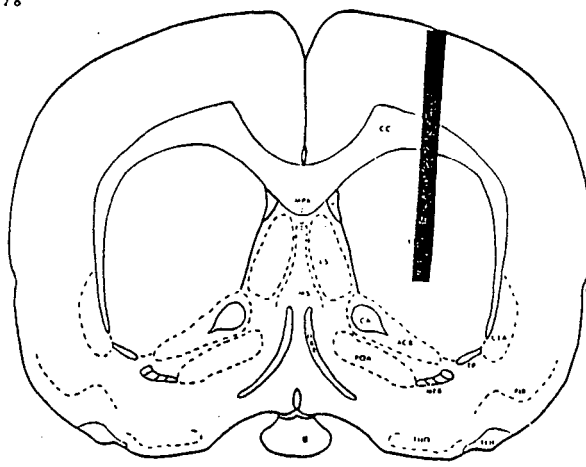
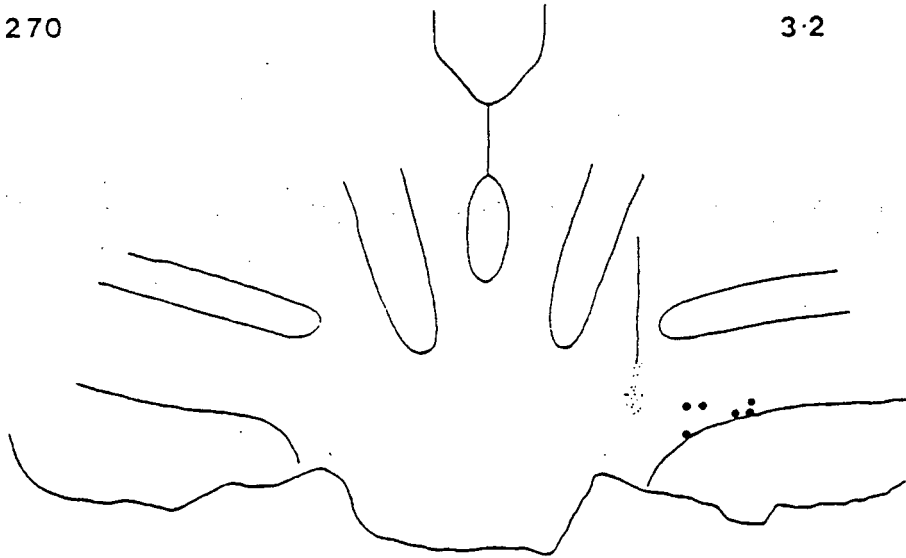


Fig. 4.23 continued.



270

3.2



2.4

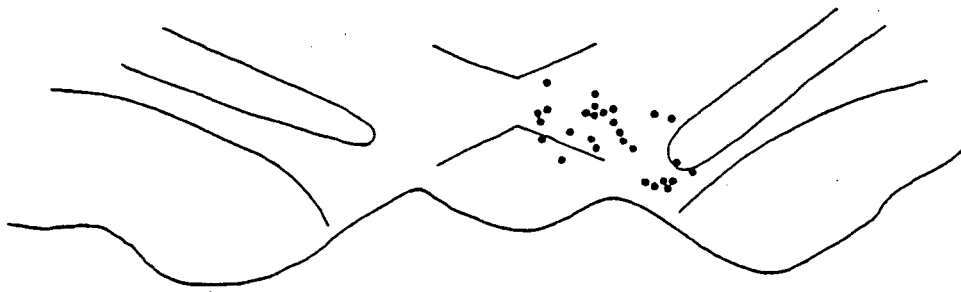
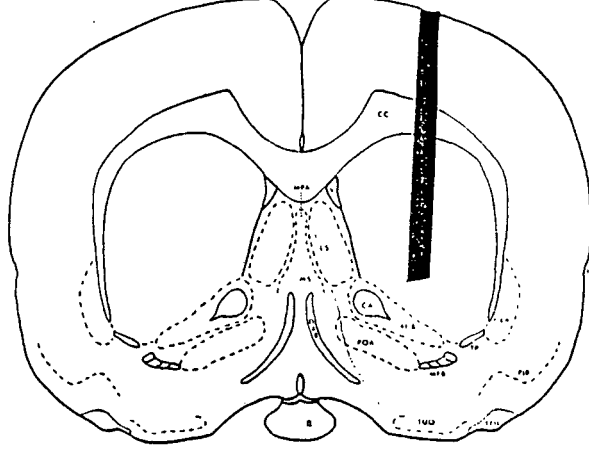
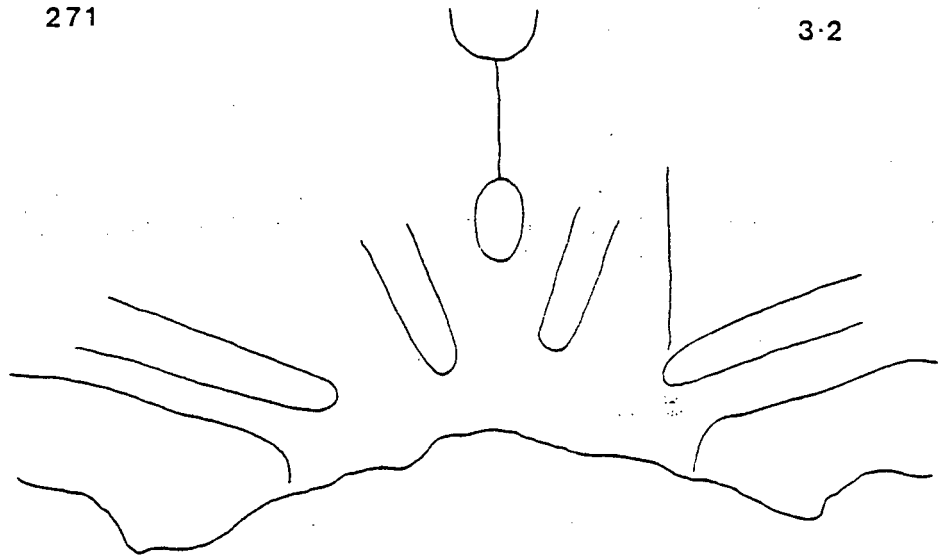


Fig. 4.23 continued.



271

3-2



2-4

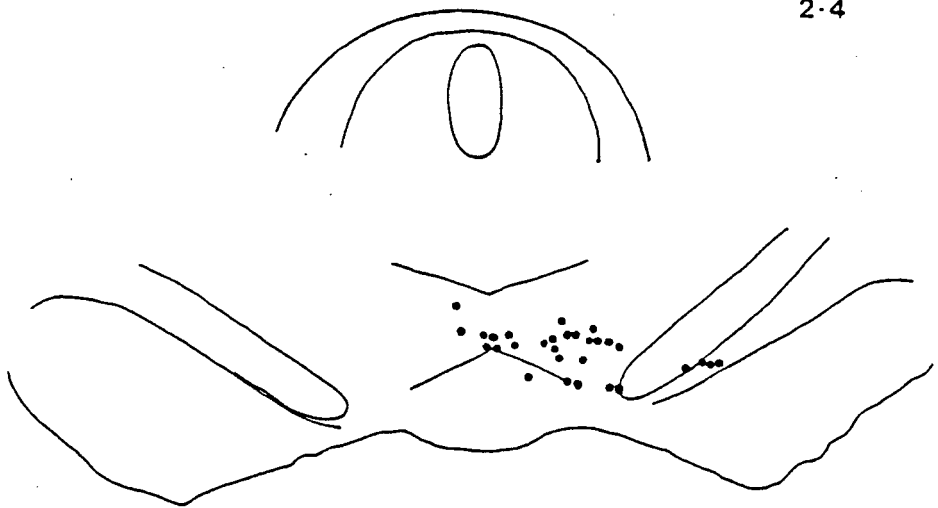
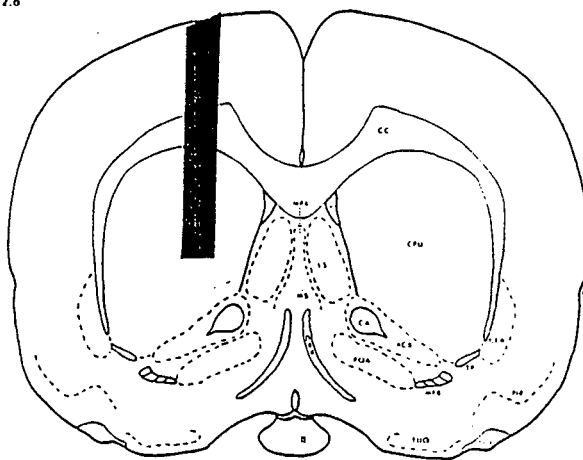
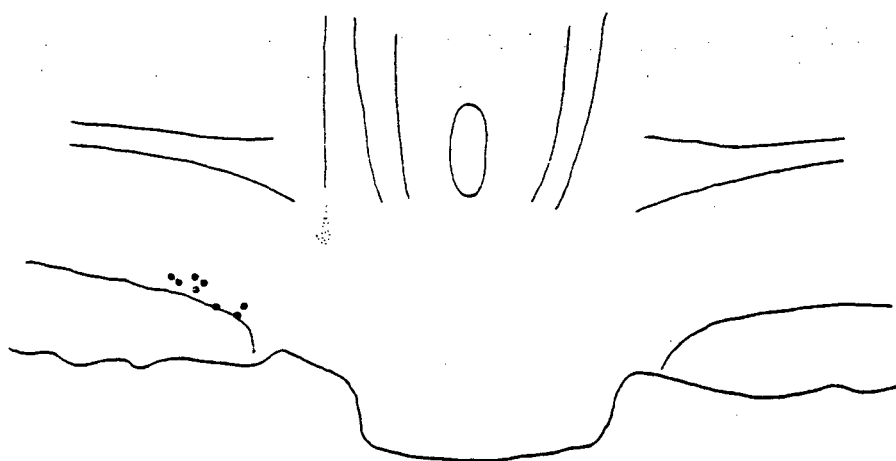


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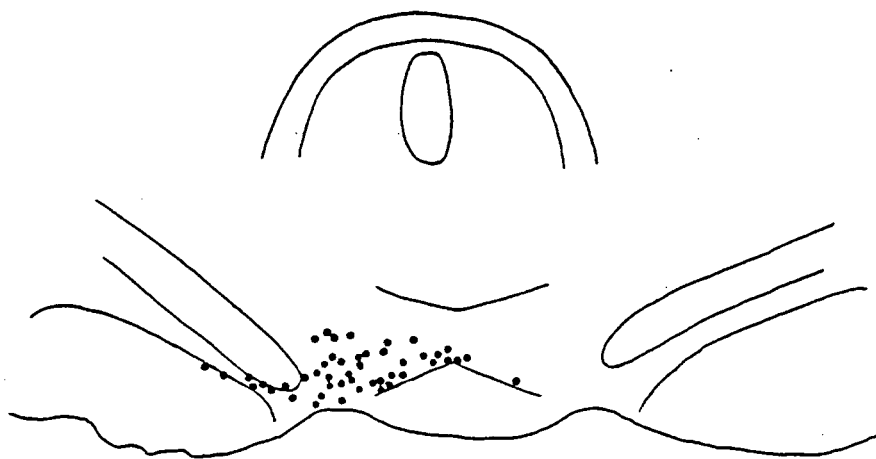
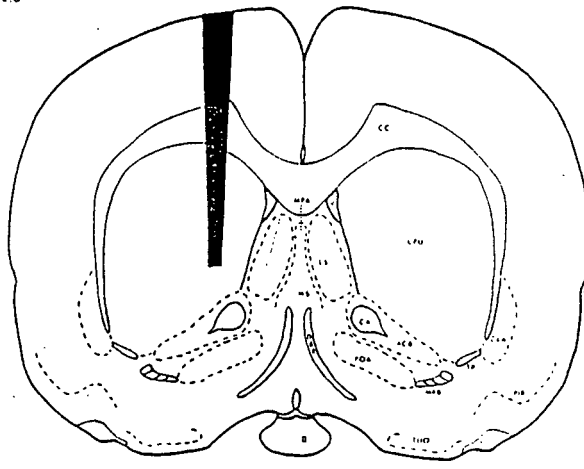
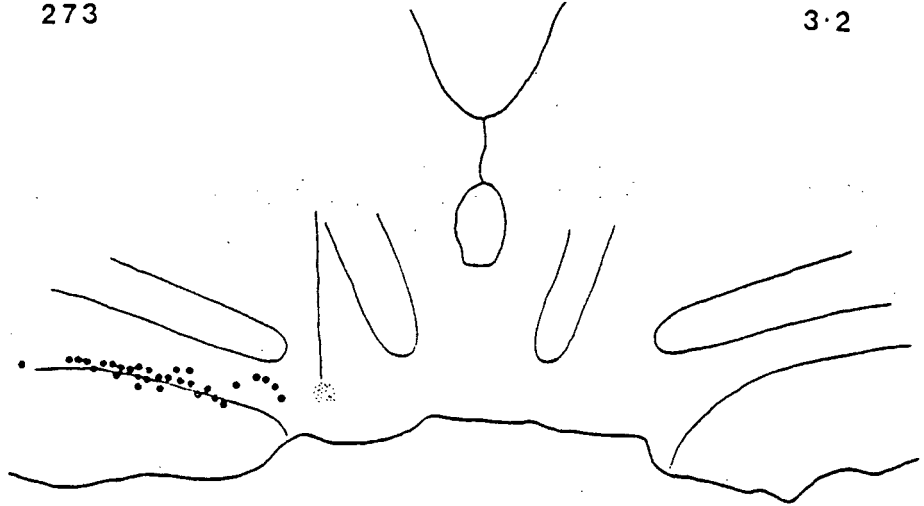


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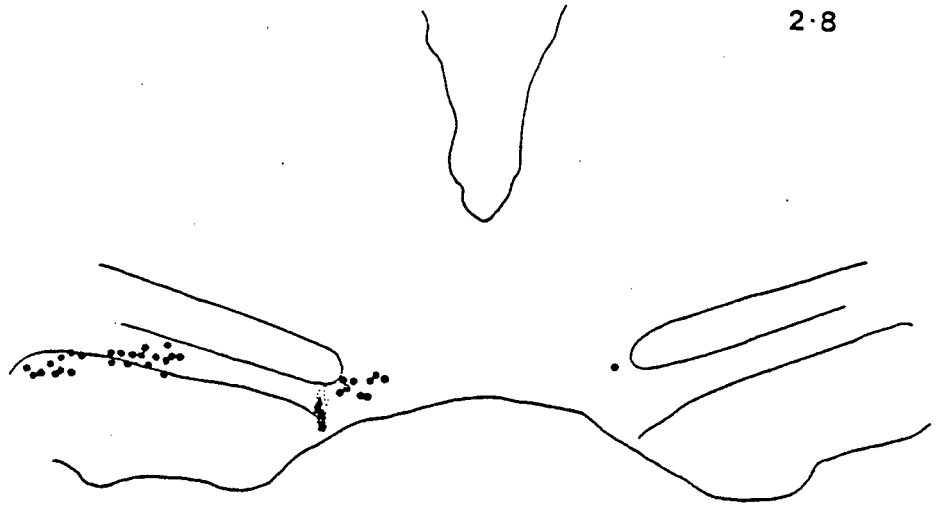


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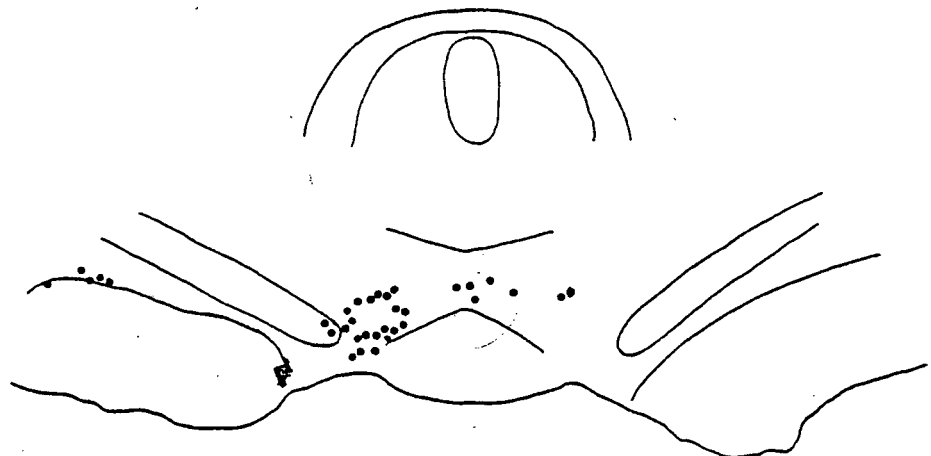
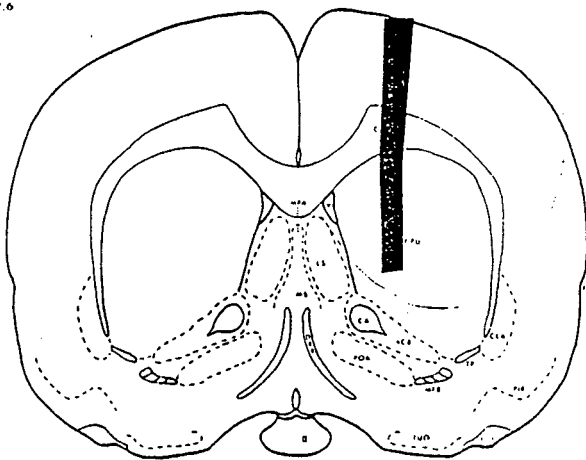
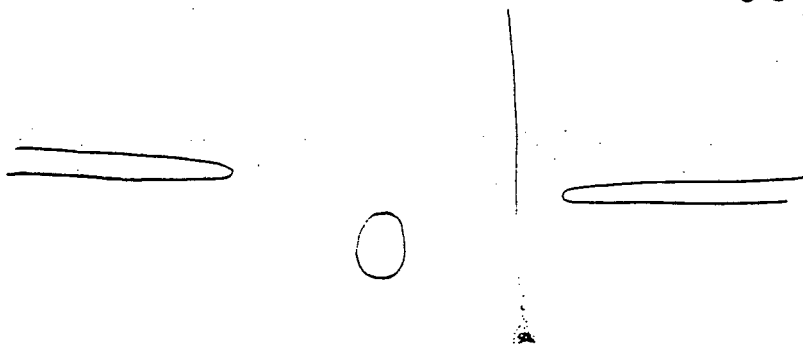


Fig. 4.23 continued.



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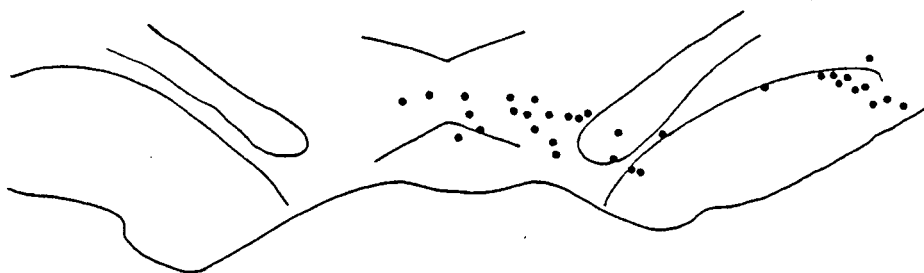
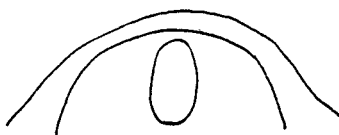
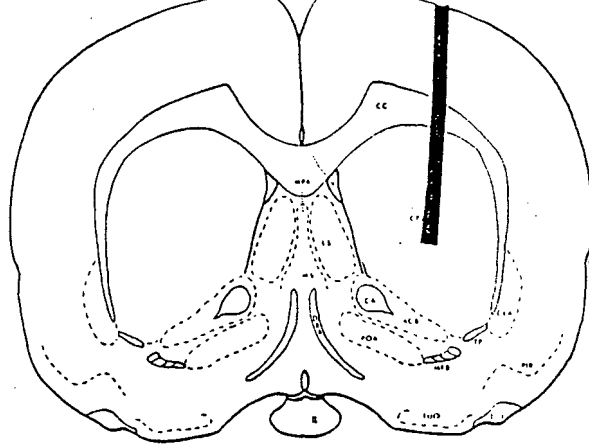
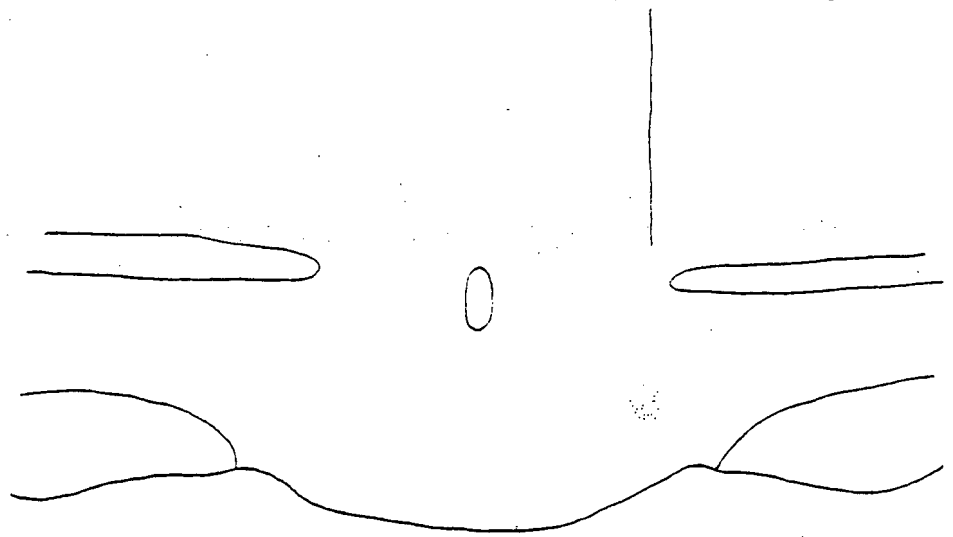


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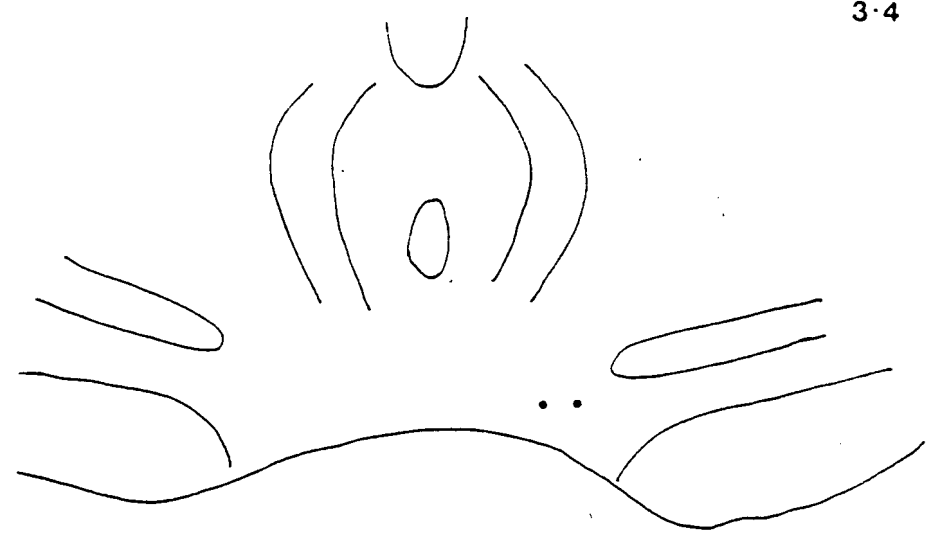


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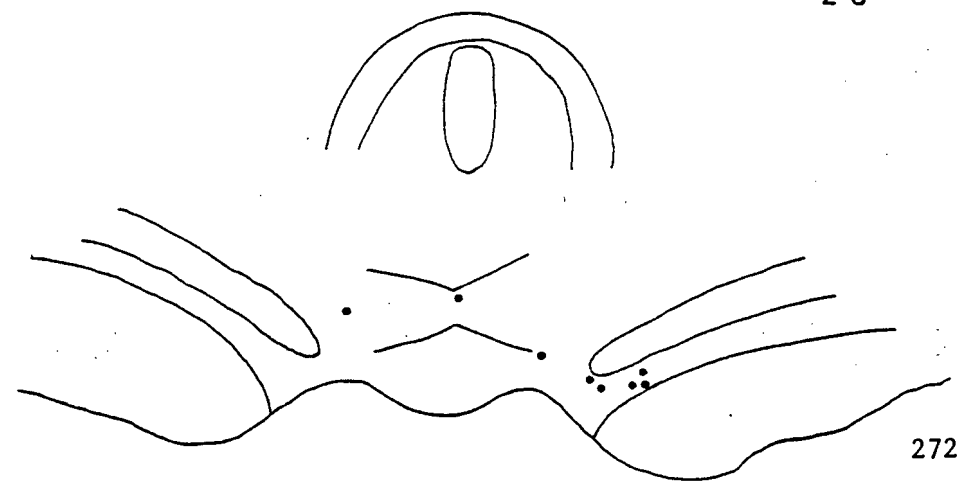
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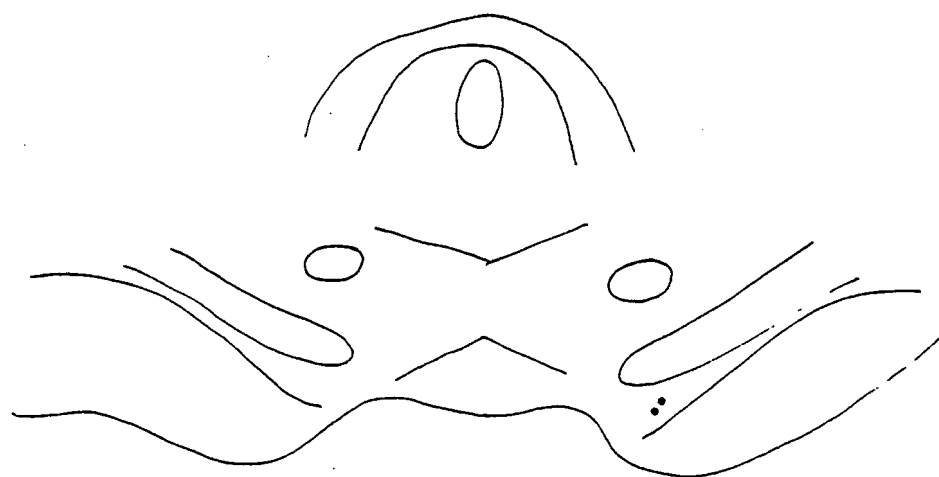
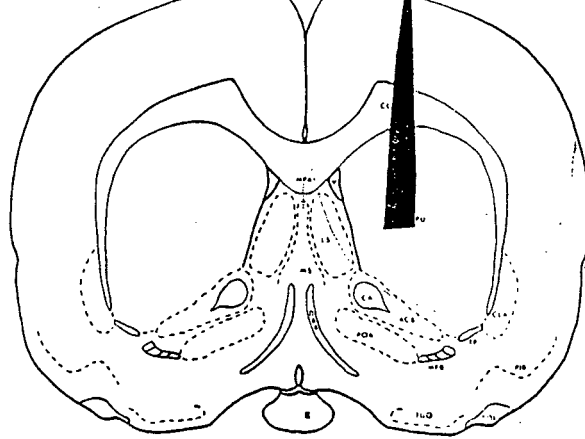
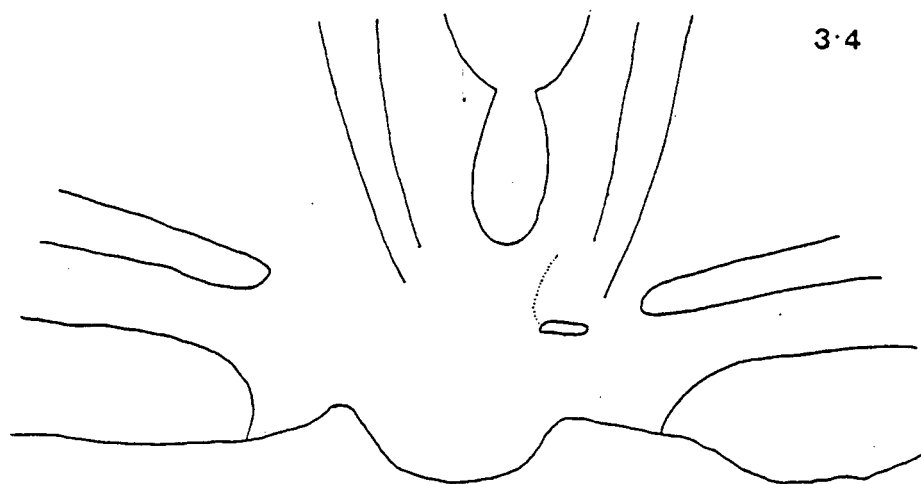
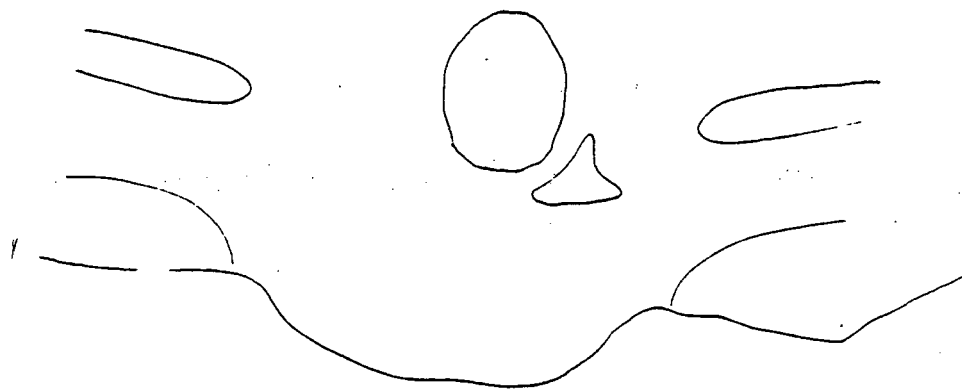


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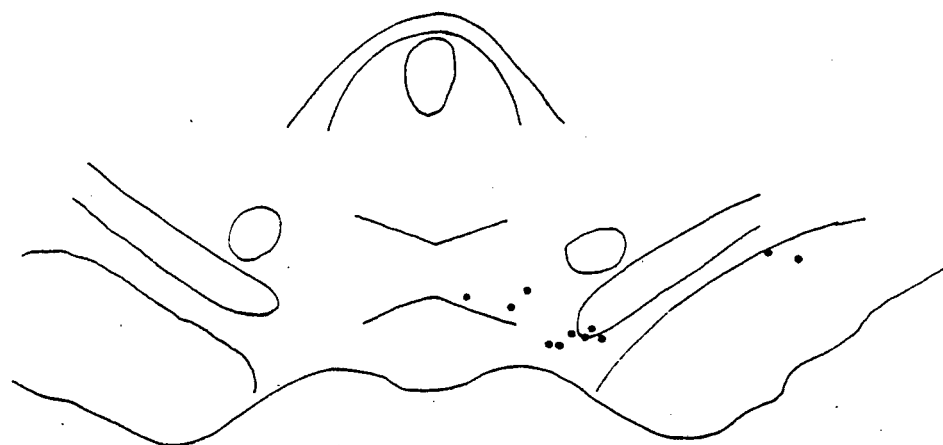


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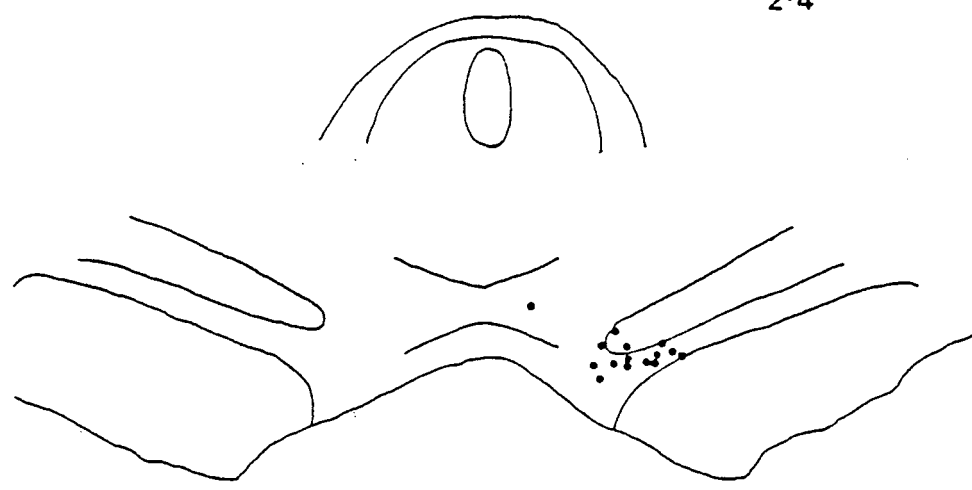


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Fig. 4.23 continued.



4.3.7 INVESTIGATION OF THE CROSSED MESOSTRIATAL PROJECTION AS A FUNCTION OF RECOVERY FROM MOTOR ASYMMETRY.

The above work delineated a lesioning zone within the ventral mesencephalon which reliably resulted in ipsilateral rotation following 6-OHDA lesioning of the dominant hemisphere with a limited dose of the neurotoxin 6-OHDA. The reason why the co-ordinates of the lesioning zone are more critical with restricted 6-OHDA doses is that the diffusional spread is limited by the small amount of vehicle. This results in greater specificity of lesion in two ways. Firstly, specificity in terms of the spatial location of the neurotoxin is enhanced, and secondly the neurochemical specificity is improved due to the lower concentration of 6-OHDA within the catecholaminergic structures of the injection zone. These anteromedial SN lesions produced depletion of the DAergic cells within the caudal and lateral SN, as well as depletion within the immediate vicinity of the neurotoxin release zone. This is due to damage to more caudal and lateral cells' rostrally projecting axons, and subsequent retrograde degeneration of these cells.

Altar et al. (1983) found that lesions of the lateral hypothalamus (LHA) with 6-OHDA produced depletion of cells retrogradely labelled with granular blue from the striatum by the same proportion as the ipsilateral striatal DA depletion. This correspondence allows an estimate of SN lesion severity to be made using retrograde tracing techniques. It is possible, thus, to

determine the number of cells remaining within a lesioned SN. One can then obtain a percent depletion index by comparing the cell count of the lesioned SN to that of intact controls. The following tables summarise the results of retrograde tract tracing with HRP performed on rats 254-277. At the end of the behavioural testing, ie. after day 32, HRP was infused into the striatum ipsilateral to the lesioned SN. One animal (262) received its HRP injection in the striatum contralateral to the lesion as a control, to test that the lesion was exclusively unilateral. A post-operative recovery time of 48 hours was allowed, and the animals were then sacrificed and processed histologically. Cell counts of the lesioned SN were made and the percent depletion for each animal was calculated.

Table 4.26. contrasts ipsilateral cell counts and the percent depletion of a lesioned SN with the presence and extent of recovery from spontaneous and amphetamine-induced rotation. Recovery is expressed as the difference between the nett ipsilateral rotation scores on days 7 and 32, divided by the score on day 7, expressed as a percentage. Recovery was indicated by a negative value, non-recovery by a positive value. The mean \pm SEM of recoverers from spontaneous nett ipsilateral rotations ($n=11$) was $54.6 \pm 2.2\%$. The mean \pm SEM of recoverers from amphetamine-induced nett rotations ($n=9$) was $44.3 \pm 2.1\%$. Non-recoverers from spontaneous nett ipsilateral rotations ($n=8$) showed an increase in nett rotations of $122.8 \pm 15.3\%$. Non-recoverers from amphetamine-induced nett rotations ($n=9$) showed an increase in nett rotations of $129 \pm 14.2\%$. HRP labelled

cell counts of the lesioned ventral mesencephalon varied from 0 to 3936 cells. Mean \pm SEM was 743 ± 55 cells, recoverers and non-recoverers. This is equivalent to a percent depletion of 100% to 47.4%. 12 had %D >90% (66.6%), 3 >80 <90 (16.6%), 2 had %D >60, <70 (11.1%), 1 had %D <50, >40 (5.5%). Mean \pm SEM is $89.3 \pm 0.73\%$. The anteromedial lesion also included the crossed projection, with the CI for lesioned subjects varying between 0 and 2.58, mean \pm SEM 0.51 ± 0.03 . This is significantly less than the CI for controls ($p < 0.001$, Mann Whitney U test).

In an attempt to answer the question whether there was a relationship between the number of cells remaining in a lesioned ventral mesencephalon and the incidence of recovery, the mean \pm SEM of ipsilateral cell counts was compared for each group, recoverers and non-recoverers. The ipsilateral cell count and percent depletion of recoverers and non-recoverers were compared (table 4.27). For spontaneous recovery the mean \pm SEM for ipsilateral cell counts and %D were 870 ± 106.3 and $87.6 \pm 1.4\%$. For spontaneous non-recovery they were 544 ± 94.1 and 92.1 ± 1.3 . While there is a trend towards non-recoverers having more depleted ventral mesencephali, this is not statistically significant ($p > 0.1$, Mann Whitney U test). For amphetamine-induced behaviour the opposite trend was apparent at first sight. The mean \pm SEM for ipsilateral cell counts and %D of recoverers were 313.9 ± 46.8 and $94.7 \pm 0.6\%$. Non-recoverers had values of 1173 ± 136 and $84 \pm 1.8\%$. The difference between ipsilateral cell counts is significant, $p = 0.02$ (Mann Whitney U test). The %D value, however, incorporates normalisation of variation in HRP density and uptake. The

difference between %D of the two groups is not significant, $p=0.1$. This suggests that behavioural recovery is independent of the number of HRP filled cells remaining within a lesioned ventral mesencephalon. The type of recovery observed was from motor hyperactivity rather than asymmetry. That asymmetry remained consistently impaired over the test period is congruent with the severity of the lesion. Recovery from motor hyperactivity may have occurred at some extra-NS site implicated in control of motor activity, eg. the limbic system.

The CI of recoverers and non-recoverers, both spontaneously and with amphetamine, was similarly depressed compared to controls. Significant recovery from motor hyperactivity could thus occur in the absence of the crossed projection. A contributory effect of the crossed projection on recovery is, however, not excluded.

Table 4.26. Table presenting the behavioural and HRP data of rats 254-270. IL=ipsilateral cell count between AP=3.6 and AP=1.8 (Pelligrino et al. 1979). %RS=percent recovery from spontaneous rotation, %RA=percent recovery from amphetamine induced rotation, %D=percent depletion of the lesioned SN, CL=cell count contralateral to the lesioned side, CI=index calculated for the side contralateral to the lesion, hrpD=factor rating the density of the HRP deposition, n=number of 50um sections. Animal 262 (*) received its HRP injection in the unlesioned side as a control.

RAT	IL	%RS	%RA	%D	CL	CI	hrpD	n
254	1396	-95	-73.1	83.5	7	0.38	9	40
255	443	-71.4	+5.6	93.1	0	0.0	7	39
256	95	-68.7	+27.6	99.4	4	0.43	5	37
257	3936	-60	+180.5	47.4	12	0.75	8	40
258	12	-7.4	-47.5	97.5	0	0	5	41
259	1798	-42.1	+79.7	74.6	8	0.53	7	43
260	0	-64.7	-64.4	100	0	0	3	39
261	112	-28.6	-52.2	87.8	2	1.0	1	39
*262	3317	+140	+16.2	9.6	1	0.12	4	39
263	223	+105	-27.9	95.8	3	0.27	6	37
264	1992	+350	+285.4	72.8	6	0.51	6	39
265	611	+33.3	+36.2	88.4	29	2.58	8	28
270	83	+260	-46.3	98.5	0	0	10	25
271	358	+50	-14.6	95.8	3	0.16	10	37
272	822	-40.6	+170.2	90.5	9	0.48	9	41
273	608	-46.7	+3800	92.8	28	1.5	10	36
275	289	+29.4	-23.8	96	10	0.97	9	41
276	244	-75	-49.7	97.8	1	0.04	10	47
277	252	+14.8	+359.7	97.1	0	0.0	9	41

Table 4.27. Table contrasting the histological findings of recoverers and non-recoverers from spontaneous and amphetamine driven rotational behaviour. RS recovery from spontaneous rotations, NRS non-recovery from spontaneous rotations, RA recovery from amphetamine driven rotations, NRA non-recovery from amphetamine driven rotations. %R percent recovery, -ve indicates recovery, +ve indicates non-recovery. IL ipsilateral HRP filled cell count. %D percent depletion on the lesioned ventral mesencephalon.

GROUP	%R	IL	%D
RS	-54.6	870	87.6
	2.2	106.3	1.4
NRS	+120.4	544	92.1
	18.8	94.1	1.3
RA	-44.4	313.9	94.7
	2.1	46.8	0.6
NRA	+549.4	1173	84
	136.1	136	1.8

4.4 DISCUSSION AND CONCLUSIONS

It is clear from the histological examination and camera lucida drawings of the lesion sites resulting from 5ul, 10ug and 2ul, 4ug of 6-OHDA that the smaller volume of 6-OHDA causes a more discrete lesion than the standard lesion. Restricted lesions are mainly of the compacta type cells of the ventral mesencephalon. Standard lesions result in non-specific damage in the form of general cellular involution and frequent vacuolation. The non-specific damage caused by the larger lesion affects SNR neurones and SNC DAergic cells. This would result in a preparation which is both striatal input (NS tract) and striatal output (SN tract) impaired, when the original intent was to induce striatal DA depletion only. Another drawback of the standard lesions was their potential for damaging the crossed projection (Douglas et al. 1984).

Standard lesions of the dominant SN induced rotation ipsilateral to the lesioned side. Amphetamine (1mg/Kg, i.p.), a DA releaser and uptake inhibitor, accentuated this effect due to DA release on the intact side. Apomorphine (1mg/Kg, i.p.), a dopamine agonist, caused rotation contralateral to the lesioned side due to its action on the supersensitive DA receptors within the striatum of the lesioned side. These results confirm those generally reported (Ungerstedt 1971, Glick et al. 1976). There was no recovery from spontaneous nett or total rotations. However, there was significant recovery from amphetamine (1mg/Kg, i.p.) induced total rotations ($p=0.02$). AI was not significantly changed over the test

period for either spontaneous or amphetamine-induced rotations with standard lesions. Since our animals recovered from amphetamine-induced hyperactivity only, our results do not confirm those of Pritzel et al. (1983) with respect to recovery from spontaneous rotation. The behaviour of our animals was monitored over a month post lesion at weekly intervals, while Pritzel et al. (1983) reported that spontaneous rotation ceased after 1 week. At this time, increased labelling of the crossed projection was observed. The temporal correlation between increased crossed projection labelling and behavioural recovery lead to the suggestion that the crossed projection was mediating the behavioural recovery. However, Pritzel et al. (1983) also lesioned the SN with kainic acid. This lesion caused increased contralateral rotation which recovered over 1 week. These animals also showed increased labelling of somata within the SN contralateral to the lesioned side. This seems paradoxical, as recovery from oppositely directed spontaneous rotation could not have been mediated by only the contralateral crossed projection. Increased activity of the crossed projection with kainic acid lesions would have increased contralateral rotation. Standard lesions can destroy the crossed projection in addition to the ipsilateral NS projection (Douglas et al. 1984). Thus, if recovery is mediated by the crossed projection, the location of the ipsilateral lesion is critical for the emergence of recovery. Therefore, we investigated the effect of restricted 6-OHDA lesions of different regions of the ventral mesencephalon on circling behaviour, in order to find a lesion location which both spared the crossed projection and induced ipsilateral rotation.

Animals with restricted lesions of the lateral and ventral SN responded similarly in that they both showed a significant increase in total rotations post lesion. There was no significant change in the direction of their rotation, which was persistently contralateral to the lesioned side. This suggests that lateral or ventral SN lesions result in spontaneous hyperactivity and hyper-reactivity to amphetamine. The reason why this should be is not immediately obvious. One possible explanation is medial and dorsal diffusion of small quantities of neurotoxin, small enough to avoid damage to the DAergic cells themselves, but sufficient to damage the sensitive DAergic dendrites, especially within the SNR. These dendrites are involved in autoregulation of the SN DAergic cells, inhibiting their firing (Cheramy et al. 1981, Jackson 1983). If their function were impaired there would be disinhibition of the NS system, with behavioural hyperactivity. Amphetamine infusion into the SN has been shown to inhibit cell firing due to DA release (Nioullon 1977). Without this autoregulatory DA release in the SN, systemic amphetamine would have an enhanced action on the nigral DAergic cells. This hypothesis cannot be verified or refuted on the basis of current data. Electron microscope analysis of SNR sections of lesioned and unlesioned animals would be one way of further investigating the question.

Lesions of the DTV induced hyperactivity to amphetamine (1mg/Kg, i.p.) administration, as shown by significantly increased total rotations. Nett amphetamine-induced rotations were also

significantly changed post lesion. Rotation was ipsilateral to the side of cannula penetration. The infusion cannula employed an oblique approach to the midline, passing through the VTN of the dominant hemisphere. Leakage of neurotoxin up the cannula track lesioned cells of the ipsilateral VTN. Thus, the animals exhibited crossing of rotation direction from the pre-lesion direction to that ipsilateral to the side of cannula penetration, although rotation was of low intensity. The hyperactivity induced by these lesions is probably due to their effect on the A10 mesolimbic projection, in agreement with the literature (Galey et al. 1977).

Lesions of the lateral SNC, ventral SN and DTV were all inappropriate for studying recovery as they did not both induce ipsilateral rotation and spare the crossed projection.

Restricted lesions of the anteromedial SNC, at the origin of the MFB, resulted in reliable ipsilateral rotation at various times post lesion. There were essentially two groups of animals according to a grouping of the results into categories as to those that recovered and those that did not. These two groupings could be sub-divided into spontaneous recoverers (n=18) and non-recoverers (n=14), and amphetamine recoverers (n=14) and non-recoverers (n=18). The same animals were not necessarily in both groups. The criterion used to determine recovery or non-recovery was the animal's rotational status 32 days post lesion compared with 7 days post lesion. If nett rotations were, for example, less on day 32 than on day 7, then "recovery" was logged. There was no minimum percent window placed on the

criterion. It is interesting to note that recoverers from both spontaneous and amphetamine-driven rotations had unchanged AI. This implies that they did not recover from a motor asymmetry, but rather from motor hyperactivity. Their percent "handed decisions" remained the same. The asymmetry index of animals in a recovery paradigm should thus always be calculated. This enables one to differentiate recovery from motor asymmetry and recovery from motor hyperactivity.

Our restricted lesions were located anteriorly within the ventral mesencephalon, at the origin of the MFB, which means that they would have damaged the crossed projection as well as the ipsilateral NS projection. The cells of the crossed projection decussate more caudally, within the DTV, and their axons would have been incorporated into the rostrally projecting ipsilateral bundle in the vicinity of the lesion site. This is confirmed by the depressed contralateral index. Recoverers from spontaneous and amphetamine-induced rotation had $CI=0.46\pm0.04$ ($n=9$), and $CI=0.31\pm0.04$, ($n=9$). Non-recoverers had similar CI. Thus, restricted anteromedial lesions affect the integrity of the crossed projection. Nevertheless, recovery does occur in the presence of these lesions, suggesting that the crossed projection does not mediate behavioural recovery.

There is reason to believe that pre-synaptic nigral events may be important in recovery. Increased striatal homovanillic acid, dopamine ratios (HVA/DA) have been measured following SN lesioning with 6-OHDA (Dravid et al. 1984). This implies that the remaining

population of ventral mesencephalic DAergic cells can upregulate their DA production. The size of this population of survivors might therefore have an important bearing on whether recovery is possible or not. A severely lesioned SN might lack the residual capacity to significantly increase striatal DA release. Thus, the extent of HRP labelled cell depletion and the status of recovery were compared. There was no relationship between the number of HRP labelled cells in the ipsilateral ventral mesencephalon and recovery or non-recovery. Recovering rats did not necessarily have more ventral mesencephalic cells than non-recoverers. Thus, recovery seems to be independent of the number of remaining ipsilateral ventral mesencephalic cells labelled with HRP from the striatum.

The question thus arises why there should be recovery of some animals and not others, and how can the independence of recovery from ipsilateral ventral tegmental cell counts be resolved? It must be noted that the recovery observed was from hyperactivity rather than asymmetry. The NS system is primarily implicated in the directed component of circling behaviour (Pycock et al. 1975). This recovery therefore implicates another system more in control of activity than asymmetry. The limbic system is an obvious candidate, as it has been shown that the n. Acc. is involved in the activity component of circling (Pycock et al. 1975). It can also be concluded that the recovery process is of a more global nature than NS recovery alone. Neurological compensation for the effects of the lesion are probably occurring at many different levels in order to compensate for striatal incompetence. Hefti et

al. (1980) have suggested ipsilateral compensation as a possible mechanism for NS recovery. Increased ipsilateral activity of the remaining neurones and striatal supersensitivity may either alone or in combination decrease the extent of NS asymmetry. However, one would then expect nigrae which are not as extensively lesioned to compensate for the NS asymmetry more readily. An anomaly appears to exist as this was not indicated by our results. A group of 4 non-recoverers with 69.5% depletion of ipsilateral ventral mesencephalic cell counts and cell count of 2280 cells showed no recovery from spontaneous or amphetamine-driven rotations. A group of 3 animals with 99.3% depletion and an ipsilateral cell count of 59 cells showed no recovery from spontaneous rotation, but a 27.7% recovery from amphetamine-induced rotation. These data indicate that a group of mildly lesioned animals did not show any recovery, while a group of more severely lesioned animals showed some recovery.

We have presented evidence that recovery and non-recovery are independent of contra- or ipsilateral nigral compensation, as determined by HRP labelling from striatal deposition sites. In particular, the crossed projection is not crucial for recovery from the motor hyperactivity component of circling, and the number of ipsilateral HRP labelled cells for recoverers is not significantly different from non-recoverers. Thus, the locus of compensation for the NS asymmetry may be extra-NS. A more global location than specifically of the NS system is suggested. Moreover, learning may be an important compensatory mechanism which would elude detection by methods such as HRP labelled cell

counts.

5.0 GENERAL DISCUSSION

5.1 INTRODUCTION

5.2 ROTATING RAT MODELS

5.3 THE CROSSED MESOSTRIATAL PROJECTION

5.4 THE EFFECT OF STANDARD AND RESTRICTED LESIONS OF THE VENTRAL MESENCEPHALON ON ROTATIONAL BEHAVIOUR

5.5 RECOVERY FROM ROTATIONAL ASYMMETRY

5.6 SUMMARY AND CONCLUSION

5.1 INTRODUCTION

Rats with unilateral 6-OHDA lesions of the NS projection display motor asymmetry in the form of rotational behaviour. The rotation is in the direction ipsilateral with respect to the lesioned side (Ungerstedt 1971). The net ipsilateral rotations decrease with time. This decrease has been interpreted as recovery from the lesion-induced motor asymmetry. Pritzel et al. (1983) have ascribed the recovery from motor asymmetry to increased activity of a crossed NS projection, which is spared by the ipsilateral lesion. As rotational behaviour is used as an index of recovery from motor asymmetry in this study, as well as others (Pritzel et al. 1983, David et al. 1984), it is important to understand the anatomical and neurochemical basis of the behaviour. Recovery is likely to be related to some aspect of the generation of circling. Thus, rotating rat models are presented in section 5.2. The anatomy of the crossed projection is relevant to the behavioural study which follows, as it is thought that the crossed projection may mediate recovery (Pritzel et al. 1983). The size of the crossed projection in normal rats was required for comparison with lesioned rats, in order to evaluate the presence or absence of the crossed projection on recovery. The size and site of decussation of the crossed projection is described in section 5.3. This information is required for the location of a restricted lesion within the ventral mesencephalon such that it induces ipsilateral circling while sparing the crossed projection. Standard 6-OHDA lesions, as used by Pritzel et al. (1983), can destroy the crossed

projection (Douglas et al. 1984). These lesions have a wide sphere of influence due to the diffusional spread of 5ul of vehicle. Thus, restricted 6-OHDA lesions of sites in the ventral mesencephalon, which are all globally affected by standard lesions, were carried out in order to find a lesion site which both induced ipsilateral circling and spared the crossed projection. The effects of standard and restricted lesions of the ventral mesencephalon on circling behaviour are described in section 5.4. The involvement of the crossed projection in recovery will be examined in section 5.5. Finally, the conclusions of the study are summarised in section 5.6.

5.2 ROTATING RAT MODELS

The first rotating rat model (Ungerstedt 1971, Jerussi and Glick 1976, Jerussi et al. 1977) was clearly defined in terms of two NS systems in competition with each other as to their respective DAergic activities. The DA release in the striatum is mediated via the topographically arranged NS projection. DA release is controlled by an inhibitory GABAergic return loop from the striatum to the dorsal SNR, where SNC dendrites are extended into the SNR. Synaptic contact between SN GABAergic and NS DAergic cells has been described (Somogyi et al. 1981). This return loop is postulated to mediate feedback control of the NS system. Local control also exists in the form of dendritic DA release from nigral dendrites impinging on DA autoreceptors on SNC dendrites and somata (Jackson and Kelly 1983). There is also local control of DA release by presynaptic autoreceptors within the terminal fields of the NS projection (Dray 1980). At the postsynaptic level the numbers of receptors and their affinities will influence the integral DAergic activity of a striatum. Combined, these factors result in a given degree of DAergic activity within the two striata. Other neurotransmitters in the striatum (eg NA and 5-HT) have been shown to have neuromodulatory roles at most. In a normal animal, there is a natural imbalance between the NS systems, with the animal preferring the side contralateral to the side of highest DAergic activity (Jerussi and Glick 1976). This will be apparent as rotation contralateral to this dominant side. A further imbalance can be created by affecting the pre- or

postsynaptic components of the NS system. Lesioning the NS tract impairs the presynaptic DA release in the striatum. Postsynaptic mechanisms of unbalancing the NS systems include unilateral striatal infusion of DAergic agonists (apomorphine) and antagonists (haloperidol).

Thus there are two rotating rat models defined in terms of their striatal state: the striatal input-impaired model, and the striatal output-impaired model. The striatal input-impaired model can be established by lesioning of the SNC DAergic cells, or by infusion of amphetamine or DA into the dorsal SNR by means of a cannula. The striatal output impaired model can be effected with a striatonigral or SNR lesion. The striatal input-impaired model is the most commonly encountered version. It has been used as an assay for both pre- and postsynaptic events. When used with a presynaptic drug, or DA releaser, rotation is evoked ipsilateral to the lesioned SN, because the DA release in the contralateral side surpasses that of the lesioned side. This model also shows a surprising result when challenged with a postsynaptically active drug, like apomorphine. In this case rotation is contralateral to the lesioned side. This result has been ascribed to a striatal phenomenon known as supersensitivity.

The supersensitivity phenomenon has been postulated to be due to DA receptor upregulation within the denervated striatum as a consequence of lesion induced striatal DA depletion or DA receptor blockade with neuroleptics (Ungerstedt 1971). However, the mechanisms of supersensitivity are by no means clear. Profound

contralateral rotation is apparent 1 day post lesion (Ungerstedt 1971), while receptor proliferation only reaches statistical significance 2 weeks post lesion. This suggests that supersensitivity is probably a function of both receptor proliferation and increased receptor affinities (Staunton 1981). It is possible that the loss of striatal terminals produces a denervation syndrome in which short term changes in receptor affinities are the most immediate compensatory response to DA depletion. Staunton, however, found no change in the affinity coefficient (K_d) accompanying or preceding receptor proliferation. K_d was measured 18 days after a 6-OHDA lesion producing 93% striatal DA depletion using the Scatchard analysis. Others have shown that DA receptor blockade with haloperidol increases the specific binding of H^3 -haloperidol to neostriatal membranes (Muller and Seeman 1977), although these results are equivocal (Von Voightlander et al. 1975). However, both DA and apomorphine depress the activity of striatal cells treated with haloperidol more effectively than controls (Siggins et al. 1976). Clearly this suggests a short term shift in NS functioning which results in an apparent supersensitivity, as shown by the contralateral circling within hours of striatal denervation.

Part of the supersensitivity to DAergic agonists might be due to a nigral-dependent metabolic effect on the SN system. DA depletion of the striatum would lead to GABA accumulation within the SN system of the lesioned hemisphere. This would result in a greater than normal nigral GABA release with subsequent DA receptor agonism, making that SN system seem supersensitive. Thus, while

the precise nature of the supersensitivity phenomenon remains to be elucidated, it is apparent that it is multifactorial, and not exclusively due to compensatory receptor upregulation.

The concept of ipsi- or contralateral circling has been used not only as a diagnostic clue as to the side of striatal DA activity, but also to the side of nigral GABA release. This introduces a third rotating rat model, one with a non-DAergic, nigral aetiology of circling. Rats rotate contralateral to the side of highest nigral GABA activity as induced by intranigral muscimol (a GABA agonist) infusion (Olianas 1978). If the SN GABA system was involved only in regulating the ipsilateral NS DAergic system, one would expect ipsilateral circling. This GABAergic circling has also been shown to be unaffected by haloperidol. If the circling was DAergic, then haloperidol would abolish it. This result confirms the existence of circling due to neurotransmission other than DAergic neurotransmission. Thus, there is another system of antagonistic influence to the DAergic system which is controlled by nigral GABA output (Olianas et al. 1978). Such a system is centred around the angular complex (AC) of the midbrain ie. periaqueductal grey and surrounding reticular formation. This introduces a fourth level of rotating rat model, those with midbrain manipulations. From this region all the component elements of the DAergic circling phenomenon can be elicited by either GABA receptor agonism or antagonism. Striatal DAergic circling must therefore involve the midbrain AC via the SNR by inhibition of SNR GABA cells. This is consistent with pharmacological studies of the various areas. Circling occurs away

from the side of highest DA activity, and striatal DA release produces nigral GABA release. This would inhibit the activity of the nigro-AC projection, resulting in a decrease in its influence at AC levels. The AC has high levels of GABA which are probably due to SNR efferents. Thus, a decrease in the nigro-AC projection's activity would result in disinhibition of the AC. If pharmacological GABA receptor blockade, equivalent to AC disinhibition, resulted in contralateral circling, this would provide strong evidence for this chain of events. The opposite effect on rotation direction would be expected with AC inhibition by GABA receptor activation. The results are as predicted. GABA receptor antagonism with picrotoxin produces slow contralaterally directed circling when injected unilaterally in the AC (Imperato and DiChiara 1981), and stereotypies when injected bilaterally. GABA receptor agonism with muscimol produces ipsilateral circling when injected unilaterally within the AC (Reaville et al. 1984), and reduced stereotypy and increased locomotion bilaterally.

The precise route whereby midbrain circling is expressed at the spinal level has yet to be determined, though the reticulospinal tracts are a likely candidate (Gonzales-Vegas 1981, Peterson and Wilson 1981).

In addition to lesion or drug-induced DA asymmetry being associated with asymmetrical motor behaviour, asymmetrical motor behaviour generates a striatal DA asymmetry. Yamamoto and Freed (1982) demonstrated that rats trained to rotate for reward developed increased DA levels in the striatum contralateral to the

direction of rotation. This DA asymmetry was temporally related to the onset of rotation, but significantly outlasted the circling behaviour. Thus the causality of DA asymmetry in asymmetrical motor behaviour has not been elucidated. It remains to be determined whether DA asymmetry causes motor asymmetry, or if it is an effect of motor asymmetry.

The circling rat paradigm has been employed to measure behavioural recovery from unilateral 6-OHDA lesions of the NS system. Pritzel et al. (1983) demonstrated that rats unilaterally lesioned with 6-OHDA in the SN recover from chronic spontaneous ipsilateral rotation within a week post lesion. Amphetamine-driven rotation ipsilateral to the lesioned side was seen as much as 90 days post lesion, although they do not describe recovery from amphetamine-induced rotation. Dravid et al. (1984) showed that rats recover from nett ipsilateral amphetamine (5mg/Kg, s.c.) driven rotations following restricted (1 and 2ul 6-OHDA) unilateral NS lesions within 22 days. Rats lesioned with larger doses (4ul) did not recover. The application of the rotation model to recovery from 6-OHDA induced motor asymmetry is essentially the following. The animal is lesioned unilaterally with 6-OHDA in the NS system. Its rotational performance is then measured at regular (typically weekly) intervals post lesion. A decrease in nett ipsilateral rotations is interpreted as indicating recovery from motor asymmetry. This behavioural recovery is then correlated with changes in the status of a known substrate of circling. The NS system is the only substrate investigated so far, as its role in circling is the most obvious and crucial. Studies have, for

example, attempted to relate behavioural recovery to biochemical changes within the lesioned NS system (Dravid et al. 1984). Pritzel et al. (1983) suggested that recovery from spontaneous rotational behaviour was mediated by the crossed projection. They attribute an observed increase in the number of HRP filled cells in the contralateral SN labelled from striatal deposition sites ipsilateral to the lesion, to reactive synaptogenesis of the crossed projection. In this study, the relationship of the crossed projection to recovery from 6-OHDA lesion induced motor asymmetry was examined, replicating (with improvements) the Pritzel et al. (1983) study. Factors like hemispherical dominance and the difference between spontaneous and amphetamine-driven rotation were taken into account and examined.

In summary, rotational behaviour is a good indicator of both DA and GABA receptor activation or blockade, with the side to which rotation occurs serving as an index of receptor agonism or antagonism. The application of the circling rat paradigm to investigating recovery from lesion-induced motor asymmetry will be discussed in section 5.4.

5.3 THE CROSSED MESOSTRIATAL PROJECTION

The crossed mesostriatal projection has been implicated as responsible for recovery from motor asymmetry (Pritzel et al. 1983). Consequently the anatomy of the crossed projection was investigated in male Long-Evans rats using retrograde HRP tract tracing from deposition sites in the striatum. The crossed projection appears as a diffuse band of HRP labelled cells from the ipsilateral medial SNC through the VTA to the DTV and contralateral VTA and SNC. Most (79.6%) of the contralateral cells were found to be between AP=2.4 and 1.0. Most (82.3%) of the cells between AP=2.4 and 1.0 fell within the DTV and VTA. The remainder were found within the SNC. As this projection arises from non-nigral structures as well as from the SNC, it is referred to as the crossed mesostriatal projection (Altar et al. 1983). The projection does not cross as a well defined fibre tract, but rather as a quasi-random dispersion of cells. The present data indicate that the crossed projection forms between 0.39% and 3.61% (1.86+-1.04%) of the ipsilateral one. The significance of the crossed projection can be questioned when considering its size. However, behaviour dependent on striatal DA receptor supersensitivity (eg contralateral apomorphine induced rotation) only becomes evident if the ipsilateral NS system is reduced to <10% of its original size. If the crossed projection escapes lesion damage, it may play an important role in the behaviour of lesioned animals.

The site of decussation of the crossed projection was of importance, because if this was known, it would help to place a NS lesion such that the crossed projection was spared. Thus, mechanical and chemical lesions were used to establish the site of decussation of the crossed projection by evaluating their effect on retrograde HRP labelling of the contralateral somata. In order to facilitate inter-animal comparison a contralateral index (CI) was calculated, which normalised the number of contralateral labelled somata. Thus $CI=0$ indicated the absence of the crossed projection, while $CI>0$ indicated its presence and extent. CI was 1.9 to 16.1 (5.8 ± 4 , SEM, $n=5$) for control animals.

Pritzel and Huston (1980) had suggested the VM thalamus as the site of decussation of the crossed mesostriatal projection. The ipsilateral SN innervates the ipsilateral parafascicular thalamus, and the ventromedial thalamus bilaterally. Midthalamic transection was thus performed with a pendulum microknife in order to test this assertion. The density and distribution of HRP labelled cells within the ventral mesencephalon in thalamic transected animals was identical to controls. More specifically, there was no attenuation of HRP labelled somata within the contralateral hemisphere as compared to controls ($CI=3.61$ to 14.85 , $n=5$). The number of contralaterally stained cells would have been expected to decrease if the crossed projection decussated via the thalamus. The lack of effect of thalamic transection on the pattern of mesencephalic cell labelling with HRP excluded thalamic commissures as possible sites of decussation. Transection of the mesencephalon in a manner identical to the thalamic transection

totally attenuated the presence of HRP labelled cells within the contralateral hemisphere (CI=0, n=5). However, these transections were somewhat gross and included mesencephalic structures other than the DTV. The dorsal tegmental and supra-mamillary decussations, and the posterior commissure were all sectioned.

6-OHDA lesions of the DTV were suggested by the mechanical lesions, and the results of HRP labelling in control animals. Most HRP labelled cells seen in the contralateral hemisphere were found within the borders of the DTV, and more laterally the VTA. The DTV is primarily a decussation of RN afferents, crossing the midline and then coursing caudally (Kappers et al. 1936). The HRP labelled cells were distributed within these fibres, with their axons crossing the midline and coursing rostrally to terminate in the striatum. The more lateral cells also had their axons decussating through the DTV. Restricted (2ul) 6-OHDA lesions of the DTV almost totally disrupted the presence of HRP labelled cells in the contralateral ventral mesencephalon (CI=0 to 1.52, n=4). These lesions were placed in the anterior half of the DTV, and were non-vacuolating. This means that they were neurochemically specific and did not damage the RN afferents. A lesion placed rostrally to the DTV, in the SMD, had no effect on the density and distribution of HRP labelled cells, which was similar to controls. Vehicle injection in the DTV did not affect the labelling of contralateral somata. This indicates that the mechanical aspect of the lesion was not responsible for attenuation of the crossed projection, but that the 6-OHDA lesioning of catecholaminergic fibres was the causative agent. It is concluded that the crossed

mesostriatal projection decussates via the DTV, and that it is catecholaminergic.

The crossed mesostriatal projection may have functional importance in a number of phenomena, including interhemispheric communication, limbic system processing, and recovery from unilateral NS lesion. As yet the projection's influence in interhemispheric communication is unknown, but an animal with a 6-OHDA lesion of the DTV would make a suitable paradigm with which to study the phenomenon. The involvement of the crossed projection in limbic system processing is suggested by the location of most of the crossed cells within the mesolimbic A10 region. Thus their function might be associated with limbic system activity. However, experimental data is still required to determine any such involvement directly. This thesis has considered only the role of the crossed projection in recovery from motor asymmetry.

5.4 THE EFFECT OF STANDARD AND RESTRICTED LESIONS OF THE VENTRAL MESENCEPHALON ON ROTATIONAL BEHAVIOUR.

Pritzel et al. (1983) have suggested that recovery from motor asymmetry is due to reactive synaptogenesis of the crossed projection. They specified recovery as decreased nett ipsilateral rotations with time. The same specification has been used elsewhere (Dravid et al. 1984, Glick and Cox 1977). In the present study, the rotational behaviour of the animals was measured in an automated rotometer. This enabled three parameters to be generated. (a) Total rotations: the number of quarter turns made, without regard to direction. This is a measure of the animals' general motor activity. (b) Nett rotations: the number of quarter turns made towards the dominant side. This represents a composite of activity and asymmetry of motor behaviour. (c) The asymmetry index: $AI = \text{nett} / \text{total} * 100$. Thus, the type of recovery could be specified on the basis of changes in each of these three parameters. Using this system, the effects of standard (5ul, 10ug 6-OHDA) and restricted (2ul, 4ug 6-OHDA) lesions of the ventral mesencephalon on rotational behaviour were tested, with the aim of investigating the role of the crossed projection in recovery from motor asymmetry.

Hemispherical dominance for rotation was established for each animal during two pre-lesion screens. The dominant hemisphere was lesioned because recovery from such a lesion is more dramatic than a non-dominant lesion. The dominant sided lesion reverses the

animal's natural asymmetry, inducing rotation ipsilateral to the lesioned side. Maximal recovery from a dominant sided lesion would re-instate the animal's original asymmetry, opposite to the lesion-induced asymmetry. Non-dominant lesions would result in an accentuation of the animal's natural asymmetry. Recovery from such a lesion would at most result in a diminution of this response. Thus, dominant side lesions optimise the rotation model for the quantification of recovery.

Standard (5ul, 10ug 6-OHDA) lesions of the dominant SN induced rotation ipsilateral to the lesioned side, effectively changing the animals' rotational asymmetry. Amphetamine (1mg/Kg, i.p.), a DA releaser and uptake inhibitor, accentuated this effect. Apomorphine (1mg/Kg, i.p.), a DA agonist, caused rotation contralateral to the lesioned side. These results confirm those generally reported (Ungerstedt 1971, Glick et al. 1976). There was no recovery from nett or total spontaneous rotations, although significant recovery from amphetamine (1mg/Kg, i.p.) induced total rotations was recorded ($p=0.02$). AI was unchanged over 32 post lesion days for both spontaneous and amphetamine-induced rotation. This is unexpected, as one would expect AI to increase due to a greater reduction in total than nett rotations. AI does increase, but only by 5.9%, not reaching statistical significance ($p>0.294$). The reason for this result is the performance of nett rotations: although nett rotations showed recovery from 201.6 ± 16.5 to 133 ± 16.3 (SEM), this decrease (34%) is not significant ($p=0.155$). Thus, these animals recovered from amphetamine-induced hyperactivity only.

Pritzel et al. (1983) did not formally test for recovery from amphetamine-induced rotation. They reported that spontaneous rotation ceased within the first post-operative week, from initial rates of 20-100 turns per hour, ipsilateral to the lesioned side. They relate the increase in HRP labelled somata within the contralateral SN of lesioned animals to the cessation of spontaneous rotation. After 90 days, the increased contralateral labelling was no longer apparent. They attribute this to reactive synaptogenesis of the ipsilateral projection taking over from the crossed projection. It is noteworthy that their 6-OHDA lesions damaged the SNR. They also employed kainic acid lesions of the SN to induce rotation. These animals displayed pronounced contralateral circling of up to 400 turns per hour, which ceased within the first post-operative week. Kainic acid would have lesioned non-dopaminergic somata within the SNR, thus inducing striatal output impairment. The 6-OHDA lesions employed in their study seem to have affected both the SNR and the SNC. Thus, for 6-OHDA both striatal input and (to a lesser extent) striatal output impairment would have resulted. Pritzel et al. (1983) found no difference between the increased contralateral labelling following 6-OHDA or kainic acid lesions. However, the former induced ipsilateral rotation, and the latter contralateral rotation. The direction of recovery is opposite for the two lesion types, yet the increase in HRP labelled cells is in the contralateral hemisphere in both cases. It is not clear how the differences between the two lesion types can be reconciled for comparative purposes.

The effects of our standard lesions agree with the observation of Pritzel et al. (1983) that standard lesions result in general SN gliosis. While standard lesions achieve DA depletion of the ipsilateral striatum to <10% of control values due to damage to the NS system (Hefti et al. 1980), they also affect other ventral mesencephalic structures like the SNR. In particular, we have shown that the crossed mesostriatal projection can be destroyed by standard lesions (Douglas et al. 1984). If recovery from motor asymmetry is mediated by the crossed projection, the location and extent of the lesion is critical for the emergence of recovery. Therefore we investigated the effect of restricted (2ul, 4ug 6-OHDA) lesions of the ventral mesencephalon on circling behaviour, in order to find a lesion location which both spared the crossed projection and induced ipsilateral rotation. The restricted lesions were more spatially specific, exhibiting less diffusional spread than standard lesions. In addition, they caused less non-specific damage such as vacuolation or general cellular involution. Restricted lesions were accurately placed in the lateral SNC, the DTV, the ventral SN (SNR), and the anteromedial SNC, at the origin of the MFB. These were discrete lesions of structures globally lesioned by standard lesions, and enabled their relative importance in inducing circling behaviour to be established.

Restricted lesions of the dominant lateral SNC resulted in an increased number of both nett contralateral and total rotations in response to amphetamine ($p < 0.05$, $p < 0.002$). The spontaneous

there were no signs of damage to the compacta type cells in the SNC or medially adjacent VTA. Nevertheless, there seemed to be an irritant effect of the 6-OHDA on the SN cells, resulting in behavioural hyperactivity. The nature of this effect might possibly be due to damage to the SNC DAergic dendrites infiltrating the SNR. These DAergic dendrites are involved in autoregulation of the activity of the NS system (Cheramy et al. 1981), and activation of their receptors results in inhibition of the NS system. Their lesion would result in disinhibition of the NS projection due to impairment of the auto-inhibitory nigral DAergic system.

Lesions of the DTV were performed using a 30 degree oblique cannula approach to the midline, thus avoiding damage to the overlying PAG and dorsal raphe nuclei. These lesions resulted in a trend towards spontaneous hyperactivity which was not statistically significant. However, amphetamine (1mg/Kg, i.p.) significantly increased total rotations ($p=0.027$). This behaviour persisted without attenuation for 24 days. Nett rotations had a tendency to deviate from the original asymmetry towards the side of cannula penetration ($p=0.288$, spontaneous; $p=0.036$ amphetamine 1mg/Kg, i.p.). Cannula penetration was consistently from the dominant side, thus there was switching of the lesioned animals' dominance. This was probably due to diffusion of 6-OHDA into the ipsilateral VTN which was penetrated by the cannula. The histology showed a 6-OHDA release zone just dorsal to the interpeduncular neurones, with a trail of involuted cells following the cannula track through the VTN. Damage to the ipsilateral VTN cells

probably resulted in the mild ipsilateral rotation. The increase in total rotations is in agreement with Galey et al. (1977), who found that lesions of the A10 region resulted in spontaneous behavioural hyperactivity. This is also further evidence for the involvement of the crossed projection in limbic processing, as most crossed projection cells were found within the boundaries of the A10 region, and A10 has been implicated in motor activity (Galey et al. 1977, Pycock et al. 1975). Whether the hyperactivity observed was due to disconnection of the hemispheres by the lesion or the resultant DA depletion of the lesioned cells' target sites is unclear. Lesions of the n.Acc, one of the major termination sites of the A10 projection, induces hyperactivity which recovers to normal levels over 8 weeks (Jackson and Kelly 1983). Thus, it is the presence of DA in the n. Acc. which is critical for normal behaviour, the hemisphere of origin being irrelevant. There are, however, inconsistencies between this observation and the hyperactivity response induced by amphetamine. If locomotor activity and n. Acc. DA levels are inversely related, and the n. Acc. is responsible for the locomotor component of circling, one would expect amphetamine to induce locomotor calming due to its effect of increasing n. Acc. DA levels. This is clearly not the case. This paradox suggests that a revision of the functional role of the n. Acc. in motor activity and circling behaviour is required.

Lateral SNC, ventral SN, or DTV lesions did not induce rotation ipsilateral to the lesioned side, and were thus inappropriate for studying recovery.

Restricted anteromedial SNC lesions did evoke crossing of rotation direction to ipsilateral to the lesioned side. These lesions were located at the origin of the MFB and produced retrograde disruption of cells lateral (within the SNC) and caudal (within the VTN) to their location, due to damage to the cells' axons. These lesions did not spare the crossed projection from damage. Thus, it was not possible to spare the crossed projection and induce ipsilateral circling with restricted lesions. This was due to the extensive cell depletion required within the ipsilateral SNC and VTN before ipsilateral circling was induced. The restricted lesions were specific for the compacta type ventral mesencephalic cells projecting rostrally to the striatum, in contrast to the standard lesions which often caused vacuolation and non-specific damage to the ipsilateral ventral mesencephalon, notably the SNR. The unilateral interruption of striatal innervation from the ventral mesencephalon caused by 6-OHDA lesions is thus the single most important factor in the generation of circling. The ventral mesencephalic cells whose unilateral lesion resulted in rotation were in the VTN and medial SNC, rather than the SNC cell cap exclusively. The recovery from net ipsilateral and total rotations, and AI, was assessed in 32 animals with restricted anteromedial SNC lesions.

5.5 RECOVERY FROM ROTATIONAL ASYMMETRY.

Decreased nett ipsilateral rotations post lesion, both spontaneously (Pritzel et al. 1983), with amphetamine (Dravid et al. 1984), or both (Glick and Cox 1978), are interpreted to indicate recovery from lesion induced motor asymmetry. Recovery from 6-OHDA induced sensory-motor asymmetry has been attributed to reactive synaptogenesis of the crossed projection, filling sites left vacant by the disappearance of terminals in the striatum of the lesioned side (Pritzel et al. 1983). Other mechanisms suggested for recovery from sensory-motor asymmetry include ipsilateral compensation of the lesioned NS system (Hefti et al. 1980, Neve et al. 1982, Dravid et al. 1984).

In this study, 56% and 43% of animals recovered from spontaneous or amphetamine (1mg/Kg, i.p.) driven rotations respectively. However, a decrease in nett ipsilateral rotations gives no definite account of asymmetry; the proportion of nett ipsilateral rotations over total rotations (AI) serves as a better index of asymmetry. Thus, when speaking about recovery, it remains to specify whether the asymmetry or activity component of circling has recovered. The following type of data was obtained in this study.

RAT	PARAMETER	TEST1	TEST2
#258	NETT	564	296
	TOTAL	595	298
#229	NETT	374	192
	TOTAL	390	264

Test 1 is performed 7 days after a restricted anteromedial SN lesion with 6-OHDA. Test 2 is performed 24 days post SN lesion. With animal #258 there is a decrease in both nett rotations and total rotations. The AI increases slightly from 95% to 99%. In animal #229 the results seem at first sight similar, but the AI changes from 96% to 73%. Animal #258 has recovered from motor hyperactivity, while animal #229 has recovered from motor asymmetry. Their nett rotational performance suggests recovery in both cases; but the changes in asymmetry and hyperactivity are intermeshed. Thus, it seems that one can only gauge changes in asymmetry by the ratio of nett to total rotations. Therefore it cannot be known whether the studies of Dravid et al. (1984) or Pritzel et al. (1983), who interpret reduced nett ipsilateral rotations as recovery from motor asymmetry, represent true recovery from asymmetry or recovery from motor hyperactivity.

Recovery from amphetamine-induced rotations may be challenged on two grounds. Firstly, there is the possibility of amphetamine toxicity. Amphetamine administration within hours after the lesion may aggravate the condition of borderline neurites which would otherwise have survived the lesion. Allowing 7 days for the lesion to stabilise might circumvent this difficulty. The significant recovery from amphetamine-induced rotation observed in this study and that of Dravid et al. (1984) support this view. The second question is that of amphetamine exhaustion (Mintz 1986). There are two pools of DA in the pre-synaptic terminals as defined by pharmacological experiments; a reserpine sensitive pool which is

DA asymmetry at the first test. Therefore, amphetamine exhaustion might mask recovery. In order to avoid this, sufficient time must elapse post lesion to allow for natural decay of DA levels within lesioned neurones from degeneration release. In this way the amphetamine effect within the lesioned hemisphere would be restricted to the surviving neurones. Pilot studies and the literature (Ungerstedt 1971) have suggested that at least a week should be allowed before amphetamine injection. Amphetamine administration before this results in profound contralateral rotation, or a weak ipsilateral response, or even a bipolar response, starting contralateral and changing to ipsilateral during the monitoring period. However, too long a stabilisation period is also not advisable in recovery experiments, because the recovery process may be missed. As it is there may be short term recovery which is not measurable with amphetamine-induced rotation. In addition to degeneration-DA-release exhaustion by amphetamine, there is also the possibility of metabolic exhaustion if large doses of amphetamine are administered in quick succession. The weekly intervals between amphetamine treatments and low dose (1mg/Kg) used in this study were considered sufficient to avoid this problem.

18/32 animals recovered from nett ipsilateral and total spontaneous rotations; $18 \pm 2/43 \pm 2$ on day 7 to $7.2 \pm 0.4/12.8 \pm 0.6$ on day 32 post lesion (nett/total \pm -SEM, $p < 0.05$, $p < 0.002$). Recovery from amphetamine-induced rotations was seen in 14/32 animals, decreasing from $262 \pm 13/307 \pm 12.7$ to $143 \pm 7.8/154 \pm 7.5$ ($p < 0.02$). The remainder of the animals were non-recoverers,

exhibiting the same or more nett rotations with time. The AI for spontaneous and amphetamine recoverers was unchanged over 32 days. The AI for amphetamine non-recoverers was significantly higher on day 32 compared to day 7 post lesion. Thus recovery, when it occurred, was from motor hyperactivity, not motor asymmetry. This recovery was similar to recovery from the amphetamine response with standard lesions, where total rotations were significantly reduced, but AI was unchanged.

The lesion locations of recoverers and non-recoverers showed no inter-group differences for spontaneous or amphetamine induced-rotation. There was thus no relationship between the location of the lesion site and the incidence of recovery.

The role of the crossed projection in recovery from motor asymmetry was investigated in 19/32 animals with restricted anteromedial SNC lesions, drawn from both the recoverers and non-recoverers described above. HRP was infused into the striatum ipsilateral to the lesioned SN and cell counts within the lesioned and contralateral ventral mesencephalon were performed. These animals had between 47.4% and 100% ipsilateral nigral cell depletion as determined by retrograde HRP labelling. They could thus be ranked as to the severity of their lesions and status of recovery. Cell counts of the contralateral ventral mesencephalon were also performed. The CI was found to be depressed for recoverers from both spontaneous ($CI=0.46\pm0.04$) and amphetamine ($CI=0.31\pm0.04$) induced rotations. Non-recoverers for spontaneous and amphetamine induced behaviour had CI's of 0.64 ± 0.13 and

0.75+-0.09 respectively. Thus, anteromedial SNC lesions affect the integrity of the crossed projection. Nevertheless, recovery does occur in the presence of these lesions, suggesting that the crossed projection does not mediate recovery.

Ipsilateral compensation represents an alternative mechanism to the crossed projection for behavioural recovery. This compensation may be pre- or post synaptic. Ipsilateral post synaptic compensation is illustrated by the enhanced rotational response to DA or its agonists that develops after unilateral damage to the DAergic innervation of the striatum (Ungerstedt 1971). This effect is thought to be mediated by a proliferation of DA receptors and enhancement of striatal DA-sensitive adenylate cyclase activity (Sato et al. 1976, but see earlier discussion). Neve et al. (1982) reported recovery of sensory-motor function parallel in timecourse to the receptor proliferation in the striatum. They concluded that this effect is the major mechanism responsible for the behavioural recovery. However, contralateral rotation to DA agonists only occurs in severely lesioned animals with >90% striatal DA (Neve et al. 1982). This process may also subserve ipsilateral NS compensation leading to behavioural recovery in these animals. However, animals with severe lesions (>90% striatal DA depletion) do not recover (Dravid et al. 1984), but it is these animals which show the contralateral rotation to apomorphine which indicates their striatal DA receptor supersensitivity. Striatal supersensitivity as a mechanism for behavioural recovery as determined by circling behaviour is thus excluded by these results.

A question not generally addressed by the literature is why there is selective recovery; why do some animals recover while others do not? Neve et al. (1983) recorded only a 50% recovery rate as judged by the return of the ability to localise somatosensory stimuli. This is similar to the recovery rate from sensory-motor asymmetry observed in the study of Kozlowski and Marshall (1983), and motor asymmetry observed in the present study. Neve et al. (1982) give no explanation for why some animals should recover and others not. Kozlowski and Marshall (1983) could differentiate recoverers and non-recoverers as the former recovered to their pre-lesion asymmetry of brain metabolism as measured by the 2-deoxyglucose (2-DG) technique. Structures showing this effect were those that normally receive DAergic innervation, ie. striatum, n. Acc., olfactory tubercle. Non-recoverers maintained their lesion induced metabolic asymmetries within the structures mentioned. The 2-DG results do not specify whether the compensation is due to changes pre- or post synaptic to the striatal DA synapse. Dravid et al. (1984) suggest a presynaptic ipsilateral effect as an alternative to the crossed projection mediating recovery. They employed reduced nett ipsilateral amphetamine (5mg/Kg, s.c.) driven rotation as their behavioural criterion for recovery from motor asymmetry. As pointed out earlier, this criterion does not differentiate recovery from hyperactivity and asymmetry. Dravid et al. (1984) account for their observed recovery in terms of ipsilateral compensation by the remaining population of nigral cells. These surviving cells were found to upregulate their DA production. They cite increased

striatal homovanillic acid, dopamine ratios (HVA/DA) as evidence of increased striatal DA turnover. Only partially (50-70% DA depletion) lesioned animals exhibited significant spontaneous recovery. If this were the major mechanism for recovery, then one would expect the DA turnover attainable by the spared nigral cells to be the limiting factor in the extent of recovery. Thus, the greater the residual capacity for DA production the greater should be the resultant recovery. A SN with a greater number of spared nigral cells might thus be expected to be associated with more complete behavioural recovery, especially with amphetamine, which would rapidly deplete a small population of spared cells. The results of the present study show that there is no relationship between the degree of sparing of the ipsilateral SN (as assessed by HRP labelling) and recovery. The percentage nigral cell depletion of control values following the lesion was $87.6 \pm 1.4\%$ for spontaneous recovery and $92.1 \pm 1.3\%$ for amphetamine recovery. The equivalent values for non-recovery are $94.7 \pm 0.6\%$ and $84 \pm 1.8\%$. These values are not significantly different.

Thus, behavioural recovery seems to be independent of both ipsilateral and contralateral observable NS repair and compensation. Consequently, behavioural recovery following nigral lesion may be a complex phenomenon incorporating extra-NS processes. It is possible that animals learn to compensate for their lesion-induced disability. Such a learning process may occur at extranigral sites (eg motor cortex). This ability may have a differential distribution throughout the rat population, just as some people are better learners than others. This may account for

why some animals recover and others do not. Another possibility is compensation at the level of the midbrain AC. If the recovery process is truly this diverse, then the rotating rat paradigm is an inadequate model with which to investigate recovery from sensory-motor asymmetry.

5.6 SUMMARY AND CONCLUSION

This study has established the following facts concerning the crossed mesostriatal pathway and circling behaviour in rats.

- 1) The crossed projection forms between 0.39% and 3.61% of the ipsilateral projection.
- 2) This crossed projection arises from ventral mesencephalic sites other than exclusively the SNC, notably the VTA and DTV. 82.3% of labelled somata in the contralateral ventral mesencephalon fell within the DTV and VTA. Thus it is termed the crossed mesostriatal projection rather than crossed nigrostriatal projection.
- 3) Cells of the crossed projection lie within the A10 boundaries, suggesting a possible functional role in limbic system processing.
- 4) The crossed projection decussates via the DTV. There is no additional crossing via thalamic commissures.
- 5) The crossed projection is susceptible to damage by 6-OHDA. Therefore it is largely catecholaminergic.
- 6) Standard (5ul, 10ug 6-OHDA) lesions of the SN result in non-specific damage of the ventral mesencephalon, notably to the SNR. The crossed projection can also be damaged by standard lesions.
- 7) The behavioural consequences of standard lesions of the SN are spontaneous ipsilateral rotation, ipsilateral rotation in response to amphetamine (1mg/Kg, i.p.), and contralateral rotation in response to apomorphine (1mg/Kg, i.p.).
- 8) Animals with standard lesions recover from amphetamine (1mg/Kg,

i.p.) induced motor hyperactivity only. There is no recovery from motor asymmetry.

9) Restricted lesions of the SN result in a lesion specific to the compacta type cells of the SNC and VTA. There is no damage to the SNR, and less diffusional spread of neurotoxin.

10) Restricted lesions of the lateral SNC do not induce ipsilateral rotation. However, they do result in increased contralateral and total rotations in response to amphetamine (1mg/Kg, i.p.). This hyperactivity does not recover over 24 days.

11) The crossed projection is spared by restricted lateral SNC lesions.

12) Restricted lesions of the ventral SN do not induce ipsilateral rotation. They result in increased spontaneous nett contralateral and total rotations. Amphetamine (1mg/Kg, i.p.) evoked the same response. This hyperactivity did not recover over 24 days. Damage to the DAergic dendrites within the SNR may account for the hyperactivity. Nigral DA release is an auto-inhibitory regulation mechanism of NS neurones. Impairment of this system may result in disinhibition of the NS projection and hence, hyperactivity.

13) The crossed projection is spared by the ventral SN lesions.

14) Restricted lesions of the DTV result in mild ipsilateral rotation due to leakage along the cannula track lesioning cells in the hemisphere of cannula penetration. They result in hyperactivity in response to amphetamine (1mg/Kg, i.p.). There is no recovery over 24 days.

15) The crossed projection is destroyed by DTV lesions.

16) Restricted lateral SNC, ventral SN, and DTV lesions are inappropriate for studying the role of the crossed projection in

recovery, as they do not both spare the crossed projection and induce ipsilateral rotation.

17) Restricted lesions of the anteromedial SNC, at the origin of the MFB, result in ipsilateral rotation.

18) 56% of the animals with anteromedial SNC lesions recovered from spontaneous nett ipsilateral and total rotations. 43% recovered from amphetamine (1mg/Kg, i.p.) induced nett and total rotations.

19) There was no recovery of AI in these animals. Thus, animals lesioned in the anteromedial SNC recovered from motor hyperactivity rather than motor asymmetry.

20) There was no relationship between the location of the anteromedial lesion and the incidence of recovery.

21) The crossed projection was destroyed by the restricted anteromedial SNC lesions. Thus, it was impossible both to spare the crossed projection and induce ipsilateral rotation with restricted lesions.

22) As significant behavioural recovery from the effects of anteromedial lesions occurred despite lesioning of the crossed projection, the crossed projection is not crucial for recovery from the motor hyperactivity component of circling. Behavioural recovery is independent of the extent of sparing of the ipsilateral SN. Thus, recovery may depend on extra-nigral processes.

We conclude the following:

(a) The crossed mesostriatal projection crossed in the DTV.

(b) It is susceptible to damage by 6-OHDA lesions of the

ipsilateral SN, which are large enough, or sufficiently critically placed to evoke a change in the dominance of rotation.

(c) Recovery is not dependent on the presence of the crossed projection. Recovery is also not dependent on the severity of the ipsilateral SN lesion. This suggests that recovery may be an extra-nigral phenomenon.

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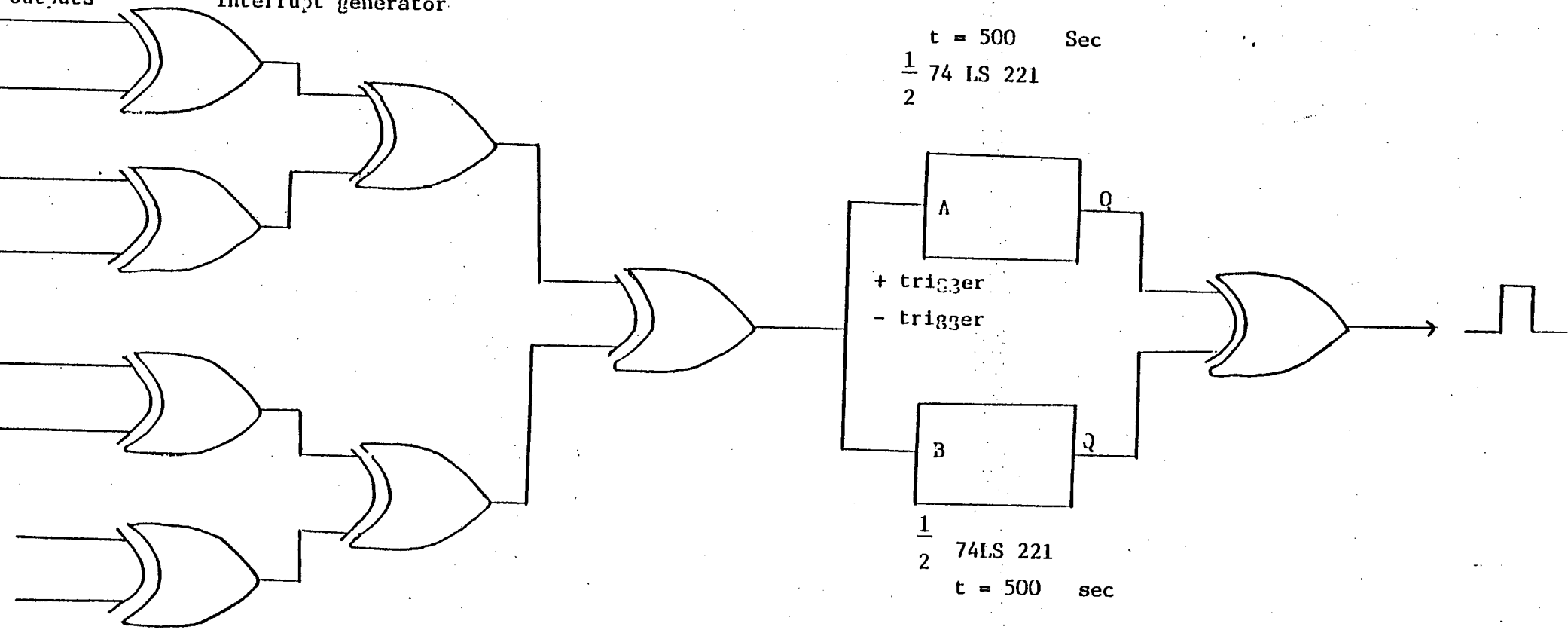
Ascending and descending projections from the nucleus reticularis magnocellularis and nucleus reticularis gigantocellularis: An autoradiographic and horseradish peroxidase study in the rat.

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APPENDIX 1 INTERRUPT GENERATOR

From
comparator
outputs

Interrupt generator



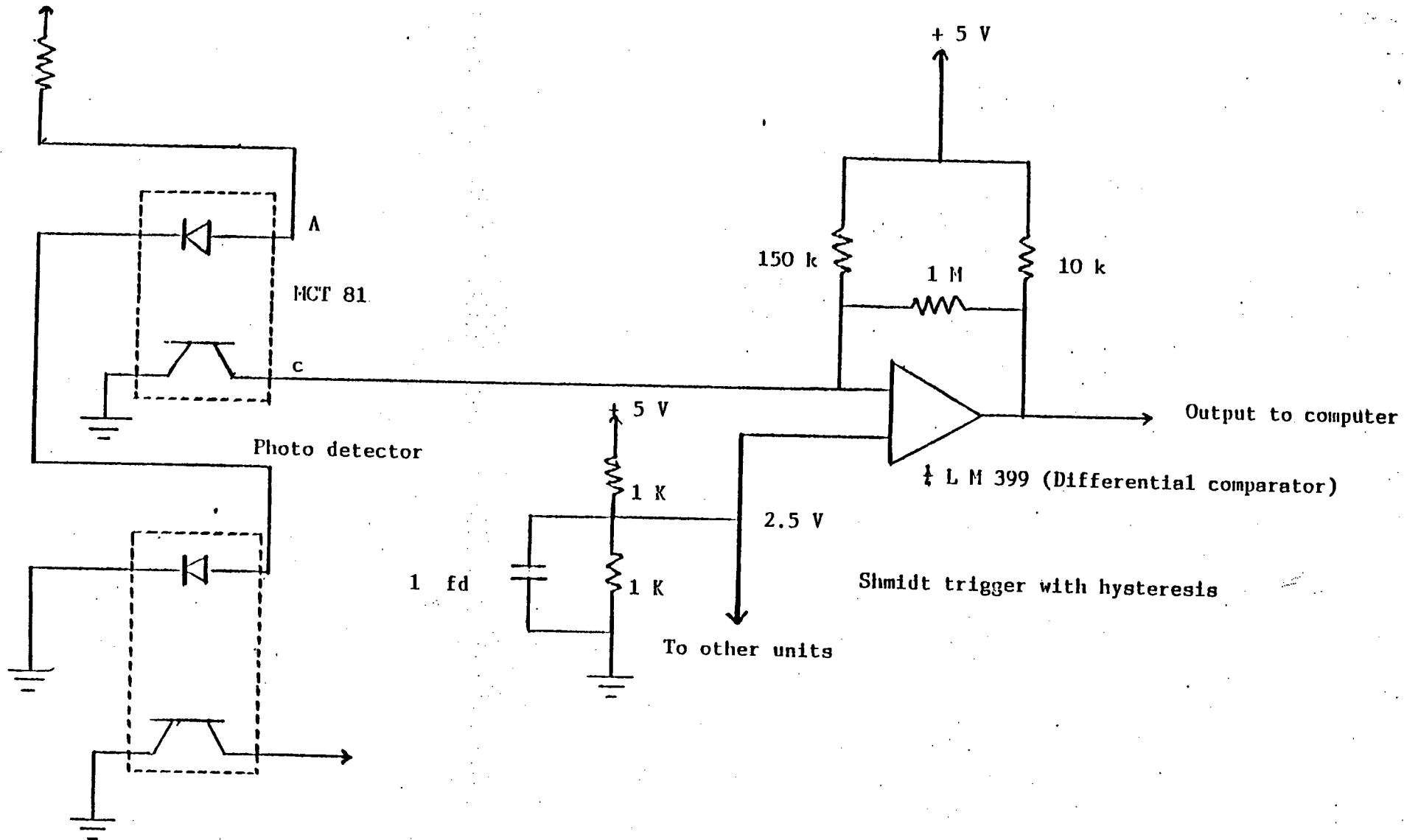
$t = 500 \text{ Sec}$
 $\frac{1}{2} 74 \text{ LS } 221$

+ trigger
- trigger

$\frac{1}{2} 74 \text{ LS } 221$
 $t = 500 \text{ sec}$

$\frac{1}{2} 74 \text{ LS } 86 \text{ Ex Or gate}$

APPENDIX 2 PHOTODETECTORS AND SCHMIDT TRIGGERS



APPENDIX 3 TABLES OF CONTROL CROSSED PROJECTION ANIMALS

Table 1.29. Cell counts of HRP labelled somata within the ventral mesencephalon, HRP (0.5 ul) deposition site rostral and caudal within the striatum.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.4	1	0	0	0	2	11	>100
3.0	2	0	0	0	2	4	147
2.6	3	6	0	1	24	68	>300
2.4	2	4	4	1	10	47	>200
2.2	2	1	2	3	22	97	>200
2.0	3	13	2	6	17	42	280
1.8	1	2	6	3	5	6	100
1.2	1	3	0	1	1	3	22
TOTAL	15	29	14	13	94	381	1307

Total ipsilateral cells IL = 1782

Total contralateral cells CL = 56

$CL/IL \times 100 = 3.1$

CI = 5.33

Table 2.30. Cell counts of HRP labelled somata within the ventral mesencephalon. HRP (0.5 ul) deposition zone located laterally and rostrally.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	0	1	1	2	8	40
3.0	1	0	0	0	7	32	40
2.4	1	2	1	1	14	29	25
2.0	1	0	0	0	3	0	35
TOTAL	4	2	2	2	13	69	140

Total ipsilateral cells IL = 222

Total contralateral cells CL = 6

$CL/IL \times 100 = 2.85$

CI = 3.0

Table 3.27. Cell counts of HRP labelled somata within the ventral mesencephalon. HRP (0.5 ul) deposition site located centrally within the caudate nucleus.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	1	0	0	0	4	0
3.2	1	0	0	0	0	0	100
3.0	1	0	0	1	0	2	>100
2.6	1	0	0	0	1	9	90
2.0	1	0	0	2	5	4	90
1.6	1	2	0	0	0	6	75
1.2	1	3	0	0	0	0	0
TOTAL	7	6	0	3	6	25	455

Total ipsilateral cells IL = 481

Total contralateral cells CL = 9

$$CL/IL*100 = 1.87\%$$

$$CI = 2.57$$

Table 4.34. Cell counts of HRP labelled somata within the ventral mesencephalon. HRP (0.5 ul) deposition site located anteriorly within the caudate (AP = 9.0 to 6.6)

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	2	0	0	1	6	25	120
3.4	1	0	0	0	3	31	80
3.2	1	0	2	0	3	32	>100
3.0	1	2	0	0	5	41	100
2.8	1	0	4	6	30	83	100
2.6	1	3	2	0	28	100	100
2.4	1	2	6	15	35	61	100
2.1	1	3	8	16	47	59	110
2.0	2	0	0	10	56	53	190
1.2	1	0	0	0	2	9	60
1.0	1	0	0	0	3	8	37
TOTAL	10	10	22	47	211	497	897

Total ipsilateral cells IL = 1615

Total contralateral cells CL = 79

CL/IL*100 = 3.6%

CI = 7.9

Table 5.33. Cell counts of HRP labelled somata within the ventral mesencephalon. HRP (0.5 ul) deposition zone rostrally located (AP = 9.0). This animal had sections extending caudally to AP = 0.0, the description of which is not included in the table, but rather included in the discussion.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	0	0	0	1	8	20
3.2	1	3	1	0	2	19	>100
3.0	1	1	0	3	8	60	100
2.6	1	2	4	1	13	88	77
2.2	1	2	4	10	55	41	86
1.4	1	2	3	2	12	12	100
1.8	1	1	1	1	0	12	52
TOTAL	7	11	13	17	91	242	535

Total ipsilateral cells IL = 868

Total contralateral cells CL = 41

CL/IL*100 = 4%

CI = 6.51

Rat 33 was sectioned beyond the level indicated in table 5. At AP = 0.6 the caudal limit of the SN was observed as 24 cells filled with HRP. 6 cells were counted in the vicinity of the oculomotor nerve. 24 cells were counted in the brachium conjunctivum and lateral lemniscus and mesencephalic reticular formation. At AP = 0.2, 9 cells were counted in the dorsal raphe and 30 in the region of the oculomotor nerve. (Whether these oculomotor nerve cells are part of the dorsal raphe spillover, or oculomotor nerve is debatable. Dorsal raphe spillover seems a likely answer as this cell group has been described to have a substantial projection to the caudate). 6 cells were observed in the reticular formation, 9 in the ipsilateral brachium conjunctivum, 2 in the contralateral brachium conjunctivum, one of which was on the border of the reticular formation. 3 cells were observed in the central tegmental nucleus. At AP = 0.0 many cells (+40) were observed in the dorsal raphe, mainly ipsilaterally, but also spreading across the midline. 4 cells were observed in the ipsilateral brachium conjunctivum and one contralaterally.

Table 6.37. Cell counts of HRP labelled somata within the ventral mesencephalon. HRP (0.5 ul) deposition site rostral (AP = 9.0), but centrally located.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.4	1	0	0	0	0	2	14
3.2	1	0	0	0	0	20	90
2.6	1	2	1	0	2	36	100
2.4	1	0	4	0	5	35	90
1.4	1	1	0	0	0	5	60
1.0	1	0	0	0	1	5	70
0.6	1	0	0	0	1	2	37
TOTAL	7	3	5	0	9	94	451

Total ipsilateral cells IL = 554

Total contralateral cells CL = 8

$CL/IL \times 100 = 1.4\%$

CI = 1.9

Table 7.Cl. Cell counts in the ventral mesencephalon between AP = 3.8 and AP = 2.0. HRP control group. HRP (2ul) deposition site ventromedially located within the caudate. The virtual injection site was located in the ventromedial region of the striatum AP = 8.0 to AP = 6.0, and labelled striatal cells extended throughout most of the nucleus except the dorsolateral quarter.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.8	1	12	0	0	0	0	0
3.6	2	75	0	0	0	0	0
3.4	4	>372	0	0	0	0	0
3.2	4	>400	16	1	0	0	1
3.0	4	>400	148	9	0	3	0
2.8	4	>400	285	8	1	1	0
2.6	2	>200	130	8	1	0	0
2.4	3	>300	245	27	2	4	2
2.2	4	>400	345	42	4	4	1
2.0	4	>400	110	27	10	10	0
TOTAL	32	>2971	1323	132	18	23	5

Total ipsilateral cells IL = >5000

Total contralateral cells CL = 46

CL/IL*100 = 0.98%

CI = 4.38

Table 8.C2. Cell counts in the ventral mesencephalon between AP = 3.6 and AP = 2.0. HRP control group. HRP (2ul) deposition site located ventromedially within the caudate.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	15	0	0	0	0	0
3.2	1	>100	3	0	0	0	0
3.0	3	>300	14	0	0	0	0
2.8	4	>400	82	5	0	1	0
2.6	4	>400	215	13	2	1	2
2.4	4	>380	220	17	4	2	3
2.2	4	>370	255	115	8	13	5
2.0	4	>320	220	178	6	17	3

TOTAL	25	>2285	1009	328	20	34	13
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Total ipsilateral cells IL = >4000

Total contralateral cells CL = 67

CL/IL*100 = 1.8%

CI = 2.0

Table 9.C5. Cell counts in the ventral mesencephalon between AP = 3.6 and AP = 2.0. HRP control group. HRP (2ul) deposition located within the ventromedial striatum. HRP halo particularly extended in this example, with spread of tracer into the ipsilateral cortex, though tracer remained confined to the ipsilateral hemisphere with especially the contralateral striatum devoid of HRP.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	0	0	0	0	4	100
3.4	6	2	0	2	7	48	>600
3.2	3	6	0	0	11	49	>300
3.0	3	0	1	2	21	88	>300
2.8	3	1	2	1	20	190	>300
2.6	3	5	1	5	57	260	>300
2.4	3	7	2	6	71	274	>300
2.2	5	5	20	38	216	388	>500
2.0	5	6	19	41	164	165	500
TOTAL	32	45	95		667	1476	3200

Total ipsilateral cells IL = >6000

Total contralateral cells CL = 172

$CL/IL \times 100 = 2.8\%$

CI = 9.77

Table 10.C6. Cell counts of HRP labelled somata within the ventral mesencephalon between AP = 3.6 and AP = 2.0. HRP control group. HRP (2.0 ul) deposition zone restricted to the dorsomedial striatum. HRP halo extending into the corpus callosum and cortex on the ipsilateral side. Dense filling of the striatum extends well caudally (AP = 6.0).

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	1	0	0	0	0	30
3.2	3	6	0	4	12	64	164
3.0	6	40	8	18	62	436	>600
2.8	4	10	12	13	135	427	>400
2.6	3	2	6	13	135	427	>400
2.4	4	8	12	123	333	400	
2.2	4	11	7	25	211	268	>400
2.0	3	9	11	41	182	152	>300
TOTAL	28	83	42	123	796	1960	2594

Total ipsilateral cells IL = >5340

Total contralateral cells CL = 248

$CL/IL \times 100 = 4.6\%$

CI = 16.1

Table 11.C8. Cell counts of HRP labelled somata within the ventral mesencephalon within AP = 3.6 and AP = 2.0. HRP deposition ventromedially located within the caudate. Limited release with diffuse striatal filling.

AP	n	2.8	1.0	0.5	0.5	1.0	2.8
3.6	1	0	0	0	0	0	53
3.4	5	0	0	0	0	6	>470
3.2	6	2	0	0	2	56	>600
3.0	5	0	0	0	4	135	>500
2.8	5	2	3	5	19	255	>500
2.6	4	2	8	3	28	287	>500
2.4	3	1	14	6	32	75	279
2.2	2	1	2	3	26	63	180
2.0	3	4	12	10	35	59	247
TOTAL	34	14	39	27	136	946	>3229

Total ipsilateral cells IL = >5000

Total contralateral cells CL = 80

CL/IL*100 = 1.8%

CI = 5.2

APPENDIX 4 SENSORY-MOTOR DESCRIPTION OF #254-#277 FOLLOWING 6-OHDA
LESION

#254

12 hours post lesion.

Left rotation, very slight responsivity to contralateral sound and whisker tweaking. Noxious olfactory stimulus induces rotation, whether applied left or right. Righting reflexes unaffected.

3 days post lesion.

Left rotation. Some contralateral deviation. Contralateral limb dragging. Contralateral neglect present. Responsivity to contralateral sound stimuli and whisker tweaking improved. Noxious olfactory stimulus on ipsilateral side evokes contralateral head turning.

7 days post lesion.

Left rotation. The animal is lethargic, doesn't seem well. The fur is fluffed up.

14 days post lesion.

Strong left posture. Left rotation. Right movement as correcting from left bias only. No right turning. Active rotation much less than before, thus animal shows strong signs of recovery. The tail shows indications of having been gnawed.

24 days post lesion

Left rotation. The animal assumes an unusual posture, with its spine stretched straight, and sniffs the air. Behaviour and responses are, however, still severely asymmetrical. No additional improvement.

32 days post lesion.

Left rotation. Animals still asymmetrical. Much the same as on day 32.

#255

12 hours post lesion.

Right rotation. Unresponsive to contralateral sound and whisker tweaking. Ear pinch doesn't evoke contralateral deviation when applied ipsilaterally. Noxious olfactory stimulus evokes rapid ipsilateral rotation. Righting reflexes are normal.

3 days post lesion.

Right rotation. Hindquarters fixed as axis, some locomotion interspersed with rotations. Better contralateral responsivity than immediately after lesion. Some left deviation both spontaneously and in response to stimuli. Noxious olfactory

stimuli presented ipsilaterally evokes some degree of contralateral deviation, but then ipsilateral rotation.

7 days post lesion.

Right rotation. Left ear twitches in response to finger-snap, but no contralateral movement results. Little or no improvement over last time.

14 days post lesion.

Right bias. Right rotation and posture. Contralateral neglect of forelimb. Rotation still chronic. Little, if any, improvement. Exploratory locomotor behaviour very poor. No left turning. No responsivity to contralateral stimuli.

24 days post lesion.

Right rotation. Circling still quite intense. Little locomotion. Axial twist present.

32 days post lesion.

Right rotation. Still no improvement, animal severely asymmetrical.

#256

12 hours post lesion.

Left rotation. Unresponsive to contralateral sound and whisker tweaking. Ear pinch does not evoke contralateral deviation. Noxious olfactory stimuli result in rapid ipsilateral rotation. Righting reflexes are normal.

3 days post lesion.

Left rotation. Some contralateral deviation, followed by ipsilateral rotation. Obstacle turning into an immovable object is present. Contralateral responsivity is not very good, but head turning in response to whisker tweak is inducible. Animal responds poorly to noxious olfactory stimulus, ie no contralateral escape deviation. Animal seems rather "depressed" and apathetic.

7 days post lesion.

Left rotation. Much the same as on previous examination.

14 days post lesion.

Left bias. Animal turns obstinately into obstruction rather than away from it. Severe lateralisation, no sign of recovery. No exploratory behaviour, contralateral limb neglect. Animal doesn't actively explore its environs.

24 days post lesion.

Left rotation. Some locomotion. Exploratory behaviour better, but still interrupted by compulsive left rotations. Axial twist is present.

#257

12 hours post lesion.

Animal locomotes, exhibits escape behaviour from noxious stimuli, whether ear pinch or noxious olfactory stimuli. No apparent deficit.

3 days post lesion.

Locomotion apparent. Maybe slight right preference. Exploratory behaviour intact.

7 days post lesion

Left and right rotation apparent

14 days post lesion

Animal active. Exploratory behaviour reasonably good. Reaction to stimuli presented on both sides.

24 days post lesion

Animal seems reasonably normal.

32 days post lesion

Right rotation apparent. Slight axial twisting, thus animal still bears slight sequelae of lesion.

#258

12 hours post lesion

Right rotation. Less responsive contralaterally. Dragging of left forelimb. Some locomotion. Hindlimb stretching. Unresponsive to contralateral noxious olfactory stimuli. Ipsilateral rotation evoked. Some contralateral deviation, though minimal.

3 days post lesion

Right rotation. Still characteristic left front forelimb dragging. No left rotation at all. Unresponsive contralaterally. Righting reflexes clumsy.

7 days post lesion

Right rotation, left forelimb dragging. Little contralateral attention, little locomotion.

14 days post lesion

Right rotation. Left forelimb dragging. Little or no exploratory behaviour.

24 days post lesion

Right rotation. No left rotation, little contralateral sensitivity.

32 days post lesion

Right rotation. Little locomotion, little contralateral attention. Righting reflexes normal.

#259

12 hours post lesion

Right rotation. Some exploratory behaviour and left head deviation. No left rotation. Contralateral noxious olfactory stimulus is noticed but there is no contralateral rotation to ipsilateral presentation. Righting reflexes fine.

3 days post lesion

Right rotation. Left forelimb dragging. Very little left deviation. Animal inattentive contralaterally. Some left head deviation but no left rotation.

7 days post lesion

Right rotation. Left forelimb dragging. Contralaterally unresponsive.

14 days post lesion

Right rotation. Limb control better and greater activity, though still severely asymmetrical.

24 days post lesion

Right rotation. Contralaterally unresponsive.

32 days post lesion.

Right rotation. Animal appears unco-ordinated, moves in "fits and starts". Rotation is compulsive. No locomotion. Some contralateral head turning in response to auditory stimuli.

#260

12 hours post lesion

Tight left rotation with stationary hindquarters. Little or no axial twisting. Contralateral sensitivity to sound. Contralateral sensitivity to noxious olfactory stimuli very poor. Ipsilaterally sensitive, but turns into stimulus. Righting reflexes poor.

3 days post lesion

Left rotation. Contralateral limb neglect. Axial twisting apparent. No escape response to noxious olfactory stimulus.

7 days post lesion

Left rotation. Some contralateral head turning. Left eye elevated. Rotation and curved posture still compulsive.

14 days post lesion

Left rotation. Some locomotion. Behaviour still compulsive with no sign of recovery.

24 days post lesion

Left rotation. Contralateral sensitivity still poor.

32 days post lesion

Left rotation. Locomotion interspersed with compulsive rotation. Exploratory behaviour better, though axial twist still apparent.

#261

12 hours post lesion.

Right rotation. Exploratory behaviour good initially. Left

deviation occurs. Animal responds to noxious olfactory stimulus by vocalising, but not withdrawing. Ipsilateral presentation results in turning into stimulus.

3 days post lesion.

Right rotation. Left forelimb dragging. Very little contralateral deviation.

7 days post lesion.

Right rotation. Left leg very extended. Grooming behaviour symmetrical.

14 days post lesion

Left and right movements, though right rotation. Contralateral responsivity poor

24 days post lesion

Right rotation. Left-sided neglect, poor responsivity

32 days post lesion

Right rotation. Left forelimb still dragging. Animal lethargic.

#262

12 hours post lesion

Hindlimb extension. Left rotation. Right forelimb neglect (dragging). Turning into immovable obstacle. Some right head deviation. Noxious olfactory stimulation produces left rotation with ipsilateral presentation, though it is noticed when presented contralaterally. Ipsilateral presentation induces turning into the stimulus; thus impaired escape behaviour. Righting reflexes fine, no hyper or hypotonicity.

7 days post lesion.

Left bias. Right quarter turns as well, locomotion improvement.

14 days post lesion.

Left rotation. Contralateral attention poor.

24 days post lesion

Left rotation. Some right deviation. Right forelimb still dragging.

32 days post lesion

Left rotation with no sign of recovery. Contralateral sensory attention poor, response to noxious olfactory stimulus

inappropriate.

#263

12 hours post lesion

Right rotation. Left forelimb dragging. Some, though little locomotion. Animal keeps its hindlimbs under its hindquarters, however, and there is no hindlimb extension. Noxious olfactory stimulus evokes inappropriate escape behaviour, but it is not noticed when presented contralaterally. Righting reflexes appropriate and quick.

7 days post lesion.

Right bias. Contralateral neglect. No exploratory behaviour. No sign of improvement. Response to noxious olfactory stimulus inappropriate. Contralateral presentation goes unnoticed, but ipsilateral presentation noticed and rotated into.

14 days post lesion

Right rotation. Animal statically rotating. Contralateral responses unimproved.

24 days post lesion.

Right rotation. Contralateral sensory impairment to auditory

stimuli and noxious olfactory presentation. Unimproved.

32 days post lesion.

Right rotation still chronic. Little exploratory behaviour. No improvement.

#264

12 hours post lesion

Left rotation. Right hindlimb extended. Forelimbs quicker, though contralateral forelimb also sluggish. Contralateral head turning in response to finger snapping. Better contralateral sensitivity to contralateral stimuli than others. Noxious olfactory stimulus presented contralaterally noticed and ipsilateral rotation evoked. Ipsilateral presentation evokes contralateral head turning but not rotation.

7 days post lesion.

Left bias, locomotion, left and right deviation. Exploratory behaviour reasonably normal.

14 days post lesion.

Left rotation with locomotion. Contralateral sensitivity impaired, but there is responsivity.

24 days post lesion.

Left rotation. Still no contralateral rotation though contralateral deviation can be evoked by stimuli.

32 days post lesion.

Left rotation. Animal still impaired. Little improvement over last observation.

#265

12 hours post lesion.

Left rotation. Right forelimb dragging. Some right head deviation. Better right body movements than others. Some locomotion. Animal vocalises upon being touched and picked up. Aggression shown to noxious olfactory stimulus (vocalising and biting). Righting reflexes normal.

7 days post lesion.

Left bias. Some right movement. Animal seems to want to move right, but then changes its direction abruptly. Curved left posture. Some locomotion.

14 days post lesion.

Left rotation. Contralateral responsivity reasonable, but contralateral rotation cannot be evoked.

24 days post lesion.

Left rotation. Some exploratory behaviour with interspersed rotation. Contralateral response to noxious olfactory stimulus inappropriate.

32 days post lesion.

Left rotation. Animal still manifests signs of lesion, little improvement.

#270

12 hours post lesion.

Right hindlimb extended. Right forelimb dragging. Animal remains poised in an extended posture for times. Some right deviation. Appropriate response to ipsilateral noxious olfactory stimulus. Contralateral noxious olfactory stimulus noticed. No fierce rotation induced, head turning away from stimulus. Righting reflexes normal.

7 days post lesion.

Left bias. Some right behaviour, attention to stimuli. Head and shoulder turning. Noxious olfactory stimulus evokes ipsilateral turn with contralateral presentation. Ipsilateral presentation evokes reasonable escape behaviour, but no contralateral turning.

14 days post lesion.

Left rotation. Some contralateral responsivity and behaviour. No contralateral rotation

24 days post lesion.

Left rotation. Animal shows some degree of locomotion. Contralateral responsivity unchanged.

32 days post lesion.

Left rotation. Locomotion present, but interspersed with bouts of chronic rotation. Animal unimproved.

#271

12 hours post lesion.

Left rotation. Hindquarters static, animal backing into turns. Right ear twitches but no contralateral head turning or rotation. Inappropriate escape from noxious olfactory stimuli or pinching. No righting reflex deficit.

7 days post lesion.

Left rotation. Activity good, inquisitive rearing behaviour. Some right attention, but of short span. Still quite severely asymmetrical. Movements do not seem fluid. They occur sequentially rather than as a series of interlinked, partly in parallel events (This observation goes for all subjects). No detection of noxious olfactory stimulus when presented contralaterally; ipsilateral presentation results in inappropriate escape behaviour. Escape behaviour thus not planned, rather a stereotypic response is elicited.

14 days post lesion

Left rotation. Exploratory movement and other parameters as above

24 days post lesion.

Left rotation. Contralateral responsivity poor.

32 days post lesion

Left rotation. contralateral behaviour and sensitivity unimproved.

#272

12 hours post lesion.

Right rotation. Inappropriate responses to noxious input. Righting reflexes normal.

7 days post lesion.

Right bias. Some contralateral attention. Animal very jittery. Sudden quick movements followed by frozen attitude, sniffing the air. Animal perambulates well. Response to noxious olfactory stimuli is correct but no contralateral rotation.

14 days post lesion

Right rotation. Little change over last report.

24 days post lesion

Right rotation. Contralateral responsivity a little better.

32 days post lesion.

Right rotation. Animal seems unimproved over last report, though better than first.

#273

12 hours post lesion

Right rotation. Left limb neglect. Some locomotion. Left head and body movements on occasion. Moves ipsilaterally if contralateral whiskers tweaked. Appropriate response to noxious olfactory stimulus but contralateral rotation.

7 days post lesion.

Right rotation. Some left choices, especially when choice width is narrow. Exploratory behaviour is good. Response to noxious olfactory stimuli is correct.

14 days post lesion

Right rotation. Contralateral responsivity no better.

24 days post lesion.

Right rotation. Animal slightly hyperactive. Stereotypic grooming behaviour.

32 days post lesion.

Right rotation. Behaviour generally unimproved over last reports.

#275

12 hours post lesion.

Left rotation. Some locomotion. Right forelimb dragging. Some element of escape behaviour from noxious olfactory stimulus, but also ipsilateral turning into stimulus.

7 days post lesion.

Left side preference. Some locomotion. Limb dragging only slight. Animal leans over left shoulder. Movements are reasonable, but a bit staccato. Not as fluid as before lesion. Response to noxious olfactory stimulus normal. No contralateral turning.

14 days post lesion.

Left rotation. Contralateral neglect better. Contralateral sensitivity about the same.

24 days post lesion

Left rotation. Animal behaves as before.

32 days post lesion

Left rotation. No improvement over previous reports.

#276

12 hours post lesion.

Left rotation. Extended right hindlimb. Right forelimb placed very far from body. Some locomotion. Noxious olfactory stimulus noticed when placed contralaterally. Ipsilateral presentation evokes contralateral head turn, but with ipsilateral rotation.

7 days post lesion.

Left side preference. Locomotion exhibited. Right forelimb seems to trail slightly.

14 days post lesion.

Left rotation. Contralateral sensitivity and behaviour as before.

24 days post lesion

Left rotation. Little or no contralateral movement other than head turning.

32 days post lesion

Left rotation. Behaviour seems little improved over previous reports.

#277

12 hours post lesion.

Left rotation. Quite active. Locomotion. Reasonably appropriate response to noxious input, though no contralateral rotation.

7 days post lesion.

Left side preference. Locomotion reasonable. Some evidence of improvement.

14 days post lesion.

Left rotation. Contralateral sensitivity improved, though contralateral rotation cannot be evoked.

24 days post lesion.

Left rotation. Animal's behaviour unchanged as compared with previous reports.

32 days post lesion.

Left rotation. Contralateral behaviour can be evoked, though body turn only, no rotation. Best recoverer so far.